A longitudinal study of brain structure and function in Rolandic epilepsy between active epilepsy and seizure remission

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PhD in Clinical Neuroscience

Abstract

Introduction

Rolandic Epilepsy (RE), an idiopathic focal epilepsy, is one of the most common epilepsies in school-age children, which frequently co-occurs with a heterogeneous mix of cognitive impairments (Pellock et al. 2016). These cognitive deficits predominantly involve reading disability, developmental language disorder and developmental coordination disorder (Overvliet et *al*. 2011, Smith *et al*. 2015, Vega *et al*. 2015). The seizures of this epilepsy spontaneously remit when the child reaches adolescence. However, it is unclear whether the same occurs with the cognitive impairments (Camfield and Camfield 2014, D'Alessandro *et al*. 1990, Garcia-Ramos 2015, Metz-Lutz and Fillipini 2006, Northcott 2006, Overvliet 2010). Furthermore, it is unknown how brain structure and function changes in respect to remission of seizures and whether this differs from the development of healthy children, which could provide a better understanding of the process to seizure remission.

Methods

In this longitudinal study, children with active Rolandic epilepsy and healthy children were invited to have structural neuroimaging and neuropsychological testing at baseline and 4.5 years later at follow-up, in seizure remission. Furthermore, at follow-up, a non-clinical sleep electroencephalogram (EEG) was performed. Details of the investigations are below:

Neuroimaging: Longitudinal structural magnetic resonance imaging (MRI) scans were used to assess cortical thickness and subcortical volumes. The *a priori* hypothesis proposed at baseline increased cortical thickness within the inferior frontal gyrus, supramarginal gyrus and inferior parietal lobe and the transverse temporal and superior temporal gyrus at baseline with reduced thinning at follow-up. Furthermore, the putamen would be larger in volume at baseline and would increase in volume at follow-up. Also included, were exploratory investigations of cortical thickness within the whole brain and parcellated regions.

Neuropsychology: Longitudinal neuropsychological assessments were used to assess fluid intelligence, single-word reading, central auditory processing, indication for developmental coordination disorder (DCD) and attention deficit hyperactivity disorder (ADHD). At follow-up, additional tasks were included to investigate phonological processing and the nature of fine-motor deficits. The *a priori* hypothesis proposed that DCD would be the most prevalent disorders, and there would be additional layers of cognitive problems. The first layer would be epilepsy specific, and the second layer suspected to be familial in origin. In seizure remission, it was proposed that the epilepsy-specific cognitive problems, which include motor and attentional issues, would reduce, whereas familial features such as dyslexia would remain.

Electroencephalography: Clinical EEG reports were reviewed, and the appearance of Rolandic spikes (RS) and their location were recorded. At follow-up, a non-clinical, sleep-deprived EEG was recorded to see if there was any evidence of RS in seizure remission. Furthermore, using global power analysis and topographic power maps, sections of awake resting-state EEG were compared with similar EEG samples from clinical EEG recordings during active epilepsy. The *a priori* hypothesis derived from Bouma *et al.* (1997), proposed that RS would be apparent in 10% of the cohort in seizure remission. An additional, exploratory component investigated changes in global EEG power and peak-dominant frequency between active epilepsy and seizure remission.

Results

Neuroimaging: The longitudinal neuroimaging data demonstrates patchy regions of cortical thinning across the cortex; however, in the areas where thinning occurred, there was a greater reduction in cortical thickness compared to controls. The greatest thinning occurred in the bilateral frontal lobes, insular and anterior temporal regions. The cross-sectional analysis shows regions of predominantly thinner cortex at baseline, whereas, at follow-up, altered regions were likely to have thicker cortex compared to controls. In particular, a large region of thicker cortex was identified around the right post-central gyrus. There was no difference in the size of putamen at baseline or follow-up, whereas the longitudinal analysis found an increase in the volume of the putamen over time. In the control group, putamen size decreased with time.

Neuropsychology: At baseline, an indication for DCD was the predominant cognitive problem; this frequently co-occurred with evidence for dyslexia and ADHD. At follow-up, the number of participants with an indication for DCD was reduced compared to baseline, but the numbers at follow-up were greater than the controls. The longitudinal analysis found an improvement in group cognition, in particular in the processing of filtered words; there was one exception, a deterioration in matrix reasoning scores. Despite group improvements, specific cognitive problems persisted in some individuals, and in some, new ones appeared in seizure remission.

Electroencephalography: The review of clinical EEG reports found a predominance for spikes over the right hemisphere. Furthermore, there were individuals with spikes before seizures and no spikes recorded during active epilepsy. At follow-up, in seizure remission, 50% had evidence of EEG abnormalities, of which, 28.6% had evidence of RS. In the remaining individuals with abnormalities, there was evidence of poorly formed, short duration, generalised spike and slow-wave discharges. Quantitative analysis of EEG resting state between active epilepsy and seizure remission found a relationship between relative delta power and time to final seizure furthermore; there was a significant difference in the topography of absolute delta power between active epilepsy and seizure remission.

Conclusions

This longitudinal controlled study has demonstrated altered cortical thickness and abnormal cognition in both active epilepsy and seizure remission. In those individuals with cognitive problems, the cognitive profile of RE is predominantly based on motor and coordination problems which co-occur in with dyslexia and ADHD. Specific cognitive problems can persist or appear in seizure remission, and thus a rethink of the educational assessment of individuals with RE is proposed. Furthermore, large amounts of thinning occur in the frontal lobes in seizure remission; this would suggest that the maturity of this structure is required for seizure remission, and this presents a new therapeutic target. The appearance of new generalised spike and waves discharges in seizure remission needs to be replicated and investigated to see how prevalent they are and whether they influence cognition. Finally, the EEG evidence lessens the role of the RS and implicates the power of resting delta wave

activity in the generation of seizures; which could improve our understanding and treatment of this common childhood epilepsy.

Acknowledgements

This thesis is dedicated to my grandmother, Anne Nicholson who sadly passed away during this project. I want to thank her for all of her support and for instilling confidence in me to achieve my goals. Further thanks to my family, who without their love and support, I would not have been able to realise this project.

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This project would not have been possible without the Waterloo Foundation and their generous funding and enthusiasm for the topic. I hope that this project has helped in creating a better understanding of this common childhood epilepsy.

Many thanks to the participants for their support and involvement in this long and arduous project. I have met many people from different backgrounds, and it is good to see that people are interested in understanding and improving the lives of people with epilepsy.

Final, thanks to everyone in the Pal, Richardson and the COSMOs lab, Prof Barker and the team at the Centre for Neuroimaging Sciences and Elka Giemsa and the team at King's College London Clinical Research Facility. Without their help and support, the administration and conduct of this research would have been laborious, breaks at the Wohl would have been boring, and I would still be getting to grips with Linux and Freesurfer. A PhD is most definitely a team effort, and I hope I have made you proud.

“Out of your vulnerabilities will come your strength.”

— **Sigmund Freud**

List of acronyms

ABR = Auditory Brainstem Response

Abx = Abnormality

ADHD = Attention deficit hyperactivity disorder

ADNI = Alzheimer's Disease Neuroimaging Initiative

AED = Antiepileptic drug

AFD = Auditory figure-ground

ANOVA = Analysis of variance

ASD: Autism spectrum disorder

B = A value used in formulating a multiple regression equation. It is an un-standardised coefficient.

BESA: Brain electrical source analysis

BJCQ = British Joint Council of Qualification

BP = Basal progenitor

B_0 = A static magnetic field

bRG = Basal radial glia

CAE: Childhood absence epilepsy

CAP = Cyclic alternating pattern

CAPD = Central auditory processing disorder

CBRS: Connor Behaviour Rating Scales

CELF: Clinical evaluation of language fundamentals

CEOP = Childhood epilepsy with occipital paroxysms

CTOPP = Comprehensive test of phonological processing

CTS: Centro-temporal spike

CVLT = California Verbal Learning Test

d = Value of Cohens d a measure of effect size

DASH: Detailed assessment of the speed of handwriting

Db: Decibel

DCD = Developmental coordination disorder

DD = Developmental dyslexia

DF = Degrees of freedom

DNPT = Differential Neuropsychological test

DTI = Diffusion tensor imaging

DWI = Diffusion-weighted image

EEG = Electro-encephalogram

EEGLAB: An interactive Matlab toolbox for processing continuous and event-related EEG, MEG and other electrophysiological data

Emx2: Homeobox protein in humans which is encoded by the EMX2 gene

EMX2: A homeobox gene which is It is expressed in the dorsal telencephalon during development in a low rostral-lateral to high caudal-medial gradient and is proposed to pattern the neocortex into defined functional areas.

ENIGMA: A international consortium which brings together researchers in imaging genomics, neurology and psychiatry, to understand brain structure and function, based on MRI, DTI, fMRI, genetic data.

ERP = Event-related potential

ESSENCE: Early symptomatic syndromes eliciting neurodevelopmental clinical examinations

FA = Fractional anisotropy

FAS = Test of verbal fluency

FDR: False discovery rate

FFT: Fast Fourier Transform

fMRI = Functional magnetic resonance imaging

FSIQ = Full scale intelligence quotient

FSL = Library of neuroimaging analysis tools for fMRI, MRI and DTI data

FWE = Family wise error

FWHM = Full width height maximum

GABA = Gamma-aminobutyric acid

GDF = Global dominant frequency

GE = Gradient echo MR sequence

Gf = Fluid intelligence within the Cattel-Horn-Carrell model of intelligence

GHE = Genetics of human epilepsy

GIN = Gaps in noise test

GM = Grey matter

GSW = Generalised spike and wave

GTCS: Generalised tonic-clonic seizure

GWAS = Genome-wide association study

ICV = Intra-cranial volume

IEC = Idiopathic childhood epilepsy

ILAE = International league against epilepsy

IIS = Inter-ictal spikes

IQ: Intelligence quotient

JHU = John Hopkins university

LKS = Landau Kleffner Syndrome

LME: Linear mixed-effects

LTM: Long Term Memory

MABC = Movement ABC

MANCOVA = Multiple analysis of covariance

MANOVA = Multiple analysis of variance

MATLAB = Matrix laboratory is a multi-paradigm numerical computing environment and proprietary programming language developed by MathWorks.

MMN = Mismatch negativity

MNI = Montreal Neurological Institute

MPRAGE = magnetisation-prepared 180 degrees radio-frequency pulses and rapid gradient-echo MR acquisition protocol.

MR = Magnetic resonance

MRI = Magnetic resonance imaging

NAD: No abnormalities detected

NEPSY = A developmental neuropsychological Assessment

Neurog2 = Transcription factor which promotes neurogenesis

NICE = National Institute of Clinical Excellence

NODDI = Neurite orientation, dispersion and density imaging

NREM = Non-rapid eye movement sleep

OASIS = Open Access Series of Imaging Studies

Olig2 = A transcription factor critical for glial cell fate determination

OSET: Organisation of Societies for Electrophysiological Technology

PAF = Peak alpha frequency

Pax6 = Protein expressed by the gene PAX6

PAX6 = During embryonic development, the PAX6 protein is thought to turn on (activate) genes involved in the formation of the eyes, the brain and spinal cord (central nervous system), and the pancreas.

PCHR = Personal child health record

PDE = Phonemic decoding efficiency

PIQ: Performance intelligence quotient

PRISMA = Preferred reporting items for systematic reviews and meta-analyses

PS: Panyiotopoulus syndrome

Qdec = Freesurfer vertices based statistical analysis software

Q-Q = Quantile-quantile plot is a probability plot

R = A free software environment for statistical computing and graphics

RAVLT = Rey Auditory Verbal Learning Test

RD = Reading disability

RE = Rolandic epilepsy

REM: Rapid eye movement sleep

RGC = Radial glia cells

ROI = Region of interest

RS = Rolandic spikes

SCAN = A battery of auditory tasks devised for the screening and diagnosis of central auditory processing disorders

SE = Spin echo MR sequence

SE = Status epilepticus

SLF = Superior longitudinal fasciculus

SNP = Single nucleotide polymorphism

SPC = Symmetrised percent change

SPLD = Specific learning disability

SPM = Statistical parametric mapping

SPSS = Statistical package for social sciences

SSD = Speech sound disorder

SSEP = Somatosensory evoked potential

STM = Short term memory

SVZ = Subventricular zone

SWE = Sight word efficiency

T = Tesla

T1 = An MR signal which reflects the time taken for the spin to return to a resting spin orientation within the main static magnetic field

TBSS = Tract based spatial statistics

TCI = Transient cognitive impairment

TD = Typical developing

TE = Time to echo

TMT = Trail making task

TOMAL = Test of memory and learning

TOWRE: Test of word reading efficiency

TR = Time to repetition

UK = United Kingdom

UNWHO = United nations world health organisation

USA: United States of America

VBM = Voxel-based morphometry

VCI = Verbal comprehension index

VEP: Visual evoked potential

VIQ: Verbal intelligence quotient

VZ = Ventricular zone

WASI = Weschler Abbreviated Scale of Intelligence

WCST = Wisconsin card sorting task

WISC = Weschler intelligence scale for children

WM = White matter

WMI = Working memory index

WRAML = Wide range assessment of memory and learning

WRAT = Wide Range Assessment Test

WRAVMA = Wide range assessment of visual-motor Abilities

X-axis= Left and right axis in neuroimaging

Y-axis= Axis between the posterior and anterior commissures in neuroimaging

Z = The number of standard deviations from the mean a data point is

Z-axis = Ventral and dorsal axis in neuroimaging

Contributions

Data collection

Baseline: Dr Colm McGinnity initiated the project. He collected clinical data information, demographics, magnetic resonance (MR) neuroimaging and some of the neuropsychological data and EEG reports. Dr Ann Smith collected neuropsychological data. EEG data collected during active epilepsy were derived from clinical EEG departments.

Follow-up: Dr Anna Smith collected some of the neuropsychological data. Prof Gareth Barker created the neuroimaging parameters derived from the legacy MR sequences. The author collected all of the follow-up neuroimaging, neurophysiological data and some of the neuropsychological data. The neuropsychological data the author collected included WASI matrices, grooved pegboard test, detailed assessment of handwriting task (DASH), developmental coordination disorder questionnaire (DCDQ'07) and Connors Behavioural Rating Scale (CBRS).

Data analysis

All of the data analyses were performed by the author.

Figure and illustrations

All are the authors own unless other sources are indicated and referenced

Signature

Ethical statement

Ethics Number: 10/H0807/093

This study had ethical approval from the Camberwell and St-Giles research ethics committee (REC). Several amendments were made to the study, and these were all ratified by the Camberwell and St-Giles ethical committee or the health research authority (HRA) in the United Kingdom.

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Publications, posters and presentations

Publications:

- Smith, A.B., Dawes, P., Smith, S. and Pal, D.K., 2017. A specific deficit of auditory processing in children with Rolandic Epilepsy and their relatives. *Epilepsy & Behavior*, *72*, pp.135-139.

Posters:

- Developmental Coordination disorder in children with Rolandic epilepsy and their siblings Smith, S. Smith, A and Pal, D.K. Presented at the International Congress of Epileptology 2017. DOI: 10.13140/RG.2.2.33060.63366

- Developmental Coordination Disorder (DCD), general coordination and fine motor deficits are prevalent in children with Rolandic epilepsy. Smith, S. Smith, A and Pal, D.K. Presented at the British Paediatric Neurologists Association Annual Conference 2018. DOI: 10.13140/RG.2.2.22994.30403

- Longitudinal MRI reveals decreased cortical thinning in Rolandic epilepsy in seizure remission: A pilot study. Smith, S. Smith, A. McGinnity, C.J. Richardson, M.P and Pal, D.K. Presented at the European Congress of Epileptology 2018. DOI: 10.13140/RG.2.2.34617.01121

- Altered cortical thinning in the left hemisphere in the Rolandic epilepsy in seizure remission. Smith, S. Smith, A. McGinnity, C.J. Richardson, M.P and Pal, D.K. Presented at the European Neuroscience Conference by Doctoral Students (ENCODS) 2019. DOI: 10.13140/RG.2.2.10830.82248

Presentations

- A systematic review of structural neuroimaging in “benign” Rolandic epilepsy. Basic and Clinical Neuroscience PhD student presentations 2017

- What remits and what remains. Presentation to the Waterloo Foundation funders 2017

- Seizure remission in Rolandic epilepsy: Calm after the storm? The Association of Neurophysiological Sciences annual conference 2018

- A longitudinal study of brain structure and function in Rolandic epilepsy between active epilepsy and seizure remission. Three-minute thesis competition. Institute of Psychiatry Student Showcase 2019.

- What remits and what remains: Investigating brain structure and function in Rolandic epilepsy in seizure remission. University of Catania Medical School 2019

Preface

This thesis presents the first longitudinal 3T structural neuroimaging study to follow children with active Rolandic epilepsy (RE) into adolescence and seizure remission. Furthermore, it incorporated a neuropsychological battery to assess features of dyslexia, developmental coordination disorder and other specific learning disabilities to explore whether these persisted into seizure remission. However, this was not the initial study design. Dr Colm McGinnity initiated the original ROLANDIC study in 2012. The ROLANDIC study was a longitudinal study which investigated children with Rolandic epilepsy and their siblings in comparison with a control group over two years. The initial data baseline data was collected, and a publication was produced “Decreased functional connectivity within a language subnetwork in benign epilepsy with centrotemporal spikes”. McGinnity C.J. *et al.* (2017). The study was redesigned after Dr McGinnity left the project. The redesign included a follow-up component in seizure remission and the removal of follow-up measurements in the sibling cohort. The redesign of the project allowed for a detailed assessment of brain structure and function in Rolandic epilepsy in active epilepsy and seizure remission and the changes between these two states, something which is lacking from the literature. Most of the data in this thesis are derived from the ROLANDIC cohorts except for the baseline neuropsychological data which included data from another study called the Genetics of Human Epilepsy (GHE) which contained data from other patients with Rolandic epilepsy. This longitudinal study was long in duration and complicated by the change in design and unexpected hiatus between baseline and follow-up. Furthermore, it involved many challenges; the principal issues were participant attrition, a change in magnetic resonance imaging (MRI) scanner between baseline and follow-up, a six-month delay to data collection of MR data at follow-up and MR movement artefact at baseline. Overall, this study presents new information, which allows for a reinterpretation of the RE literature.

Contents

Figures

Tables

Equations

1 Introduction

Rolandic epilepsy (RE) is a non-lesional (idiopathic) focal epilepsy seen in school-age children which can be associated with cognitive impairment. In the UK, approximately 700-800 new cases are expected each year (Mellish *et al.*, 2015). Furthermore, worldwide, it is believed to be the most prevalent childhood epilepsy (15% of all new cases) (Camfield and Camfield, 2002; Watanabe, 2004). Routine brain imaging rarely detects any abnormalities, and incidental findings are comparable to those in migrainous or normal children (Boxerman *et al.*, 2007). Historically, children with RE were said to have a normal neurological exam (Fejerman and Caraballo, 2007; Panayiotopoulos *et al.*, 2008). However, despite the apparent "benign" nature of the disorder, many children with RE exhibit cognitive problems.

In RE, a significant proportion of children present with cognitive and behavioural problems; before, or following their diagnosis, which has no relation to the number of seizures suffered (Overvliet et al. 2011b, Vega et al. 2015). These findings prompt concern in parents and teachers as they consist of a spectrum of deficits involving speech and language, hearing, reading, visuospatial skills, memory, attention and motor function (Vannest et al. 2015). These deficits can vary in severity with the worst cases evolving into a disorder with developmental regression (Gobbi et al. 2006). Furthermore, there is evidence to suggest that cognitive impairment is still apparent, to a lesser extent, after seizures have remitted (Deonna et al. 2000, Northcott et al. 2006, Monjauze et al. 2011b). It is suspected that cognitive impairment may be linked with subtle changes in brain structure.

Recent studies investigating brain structure in RE have produced evidence implicating differences in cortical thickness compared to healthy controls. These changes have been documented around the rolandic fissure lateralised with the EEG inter-ictal spikes but also globally across the cortex and in subcortical regions (Lin et al. 2012, Overvliet et al. 2013a, Pardoe et al. 2013, Garcia-Ramos et al. 2015). White matter changes have also been documented in the connections of the pre and post-central gyrus but also extensively outside of these regions predominantly in the longitudinal fasciculus and corpus callosum (Ciumas et al. 2014, Kim et al. 2014, Xiao et al. 2014). Longitudinal studies

show that grey and white matter conceivably have delayed maturation compared to healthy children. It is unclear whether the brains structure normalises with remission of seizures and whether the changes are causal or as a result of seizures and spikes.

Seizures in RE are age-limited with most children experiencing several seizures. The first seizure can occur between two to twelve years of age (with a clear peak at seven to nine years) followed by remission usually before age sixteen (Lüders et al. 1987, Dalla Bernardina et al. 2005). In 80% of cases, seizures are infrequent (2-10 in their lifetime). Within this infrequent group, 10% will present with a single seizure (Stephani 2001). Twenty per cent of children, mostly younger, will have many seizures with reports of several per day leading to hundreds of events (Peters et al. 2001, Kramer et al. 2002, Dalla Bernardina et al. 2005). Most children with RE (65-70%) experienced seizures during sleep, or upon awakening, a few (10-20%) have diurnal only seizures, and the remaining have both (Degen and Rodin 1991). The range of seizure onsets if quite well defined, whereas seizure frequencies and semiologies are less defined and atypical manifestations are common.

In RE, seizures have distinct semiologies which include unilateral facial sensorimotor symptoms in 30% of patients; oro-pharyngo-laryngeal symptoms in 53%, speech difficulties in 40% and hypersalivation in 30% (Beaussart 1972). Despite their distinct semiology, around 20-25% of children will experience more than one type of seizure, and atypical features are common (Loiseau and Duché 1988, Wirrell et al. 1995). Atypical features include status epilepticus, screaming, auditory hallucinations, visual impairment, vomiting and severe pain (Wirrell et al. 1995, Stephani 2001). Both the common and uncommon ictal features involve a mix of positive and negative sensory, motor and autonomic phenomena. These features convey the activation of distinct cortical and subcortical regions. A similar variation of distribution is seen in the location of inter-ictal spikes (IIS).

An inter-ictal spike is an electrographic representation of cortical hyperexcitability, and there is a collection of evidence indicating a genetic origin with an associated cognitive risk (Shewmon and Erwin 1988, Smith et al. 2012b, Panjwani et al. 2016). Segregation analysis has revealed autosomal dominant inheritance of centro-temporal spikes (CTS) in families with RE probands. (Bali et al. 2007). It is suspected that these spikes affect cognitive ability. However, siblings and parents of probands may exhibit the same cognitive problems with or without CTS (Bali et al. 2007, Clarke et al. 2007, Smith et al. 2012b, Verrotti et al. 2013).

Even though centro-temporal spikes are seen as a “hallmark” of RE they are not exclusive to this epilepsy, they can be seen in many disorders, particularly those of neuro-developmental origin. They occur in idiopathic epilepsies such as; Panayiotopoulos (Covanis et al. 2003) and Landau-Kleffner syndromes (LKS) (McVicar and Shinnar 2004) and neurological disorders such as migraine (Kinast et al. 1982). They are apparent in epilepsies of presumably symptomatic origin; such as in cerebral palsy (Degen et al. 1999) or as the result of brain lesions (Santanelli et al. 1989). Their appearance can also be seen in genetic disorders with severe cognitive deficits such as Rett syndrome (Niedermeyer and Naidu 1990) and Fragile X (Musumeci et al. 1988) and in other neurocognitive disorders such as attention deficit hyperactivity disorder (ADHD) (Holtmann et al. 2003), developmental coordination disorder (DCD) (Scabar et al. 2006) and autism (Chez et al. 2006). Finally, they are also evident in normal, presumed healthy children (2-3 %) (Eeg-Olofsson et al. 1971, Gertler and Stack 2015, McNally and Kossoff 2015) either spontaneously or with tactile stimulation (Langill and Wong 2003). This diversity makes RE quite difficult to study as CTS are far from a “hallmark” of RE; what defines RE is its seizure semiology.

The seizure features which define Rolandic epilepsy are its semiologies and age limitation. In comparison, the frequency of seizures and when they occur in the circadian cycle is not well defined. Inter-ictal spikes have a limited role in defining RE as they can appear in many cortical locations while awake or in a sleep state and thus can only support a diagnosis of RE. Finally, in individuals with RE, there is a diversity of cognitive profiles of varying severity, which suggests a heterogeneity of phenotype. The diversity of cognitive problems requires further investigation to define the epilepsy syndrome better.

Understand the cognitive profile of children with RE requires an investigation of the phenomena from several angles. First, does RE have a distinct profile from other focal and generalised idiopathic epilepsies or is it part of a general cognitive issue in children with epilepsy. Two, if the cognitive problems are distinct, are they seen in siblings or other family members. Three, if there are variations in cognition between children with RE and their families could this be due to the role of spikes and seizures. Four; to identify which cognitive problems are epilepsy specific, requires an investigation of cognition in seizure remission. Therefore, this review aims to present evidence of cognitive problems in the idiopathic epilepsies of childhood (both focal and generalised). The main body of the text will

focus on cognition in Rolandic epilepsy during active epilepsy and seizure remission. It will state evidence derived from neuropsychological data and where possible back up these findings with neurophysiological data. This review will be used to develop robust hypotheses which can be tested in this PhD.

2 Evidence for cognitive dysfunction in Rolandic epilepsy

2.1 Cognition in epilepsy

Cognitive problems can be apparent at the time of diagnosis or even antedate the first recognised seizures, which implies a relationship between the epileptogenic process and cognition (Oostrom et al. 2003, Berg et al. 2005, Berg et al. 2011, Hermann et al. 2012, Jackson et al. 2013). To put this into context, around 24% of children will have received academic help before they receive an epilepsy diagnosis (Berg et al. 2011). The risk of impaired cognition in epilepsy can be related to the age of onset (Kaaden and Helmstaedter 2009), aetiology (Besag 2006), epileptiform discharges (Aldenkamp and Arends 2004, Ebus et al. 2012) and psychiatric and behavioural problems (Cornaggia et al. 2006). Moreover, cognition can also be altered, following seizure onset, by the duration of epilepsy (Elger et al. 2004) and pharmacological interventions (Lagae 2006). All of these problems are difficult to disentangle as some or all of these factors could play an important role. Similar to the risk of cognitive dysfunction, there is variation in the severity and occurrence between or within children with epilepsy.

The severity and appearance of cognitive problems in children with epilepsy are diverse. They can be time-sensitive, appearing transiently (Binnie 2003), chronically (Elger et al. 2004) or in the extreme, progressively (Seidenberg et al. 2007). Furthermore, the severity of these problems varies within and between epilepsy syndromes suggesting that these deficits are multi-factorial (Oostrom et al. 2003, Elger et al. 2004, Callenbach et al. 2009). Finally, in some children with epilepsy, there is no evidence of cognitive deficits, and they can perform to the highest of academic levels. This evidence is only hinted at in the literature but can distort group analyses (Berg et al. 2008a, Reilly and Neville 2011). Høie *et al.* investigated 198 children with epilepsy: 25% had idiopathic epilepsies, and another 24.7% had cryptogenic epilepsies. When assessed using Raven's matrices (non-verbal intelligence), there was a large variation in abilities. A large proportion of the group was in the 10th percentile; the individuals were classified as having “mental retardation”, presumed to be due to symptomatic lesions yet around 40% were achieving average to above average scores (50 to 95 percentile).

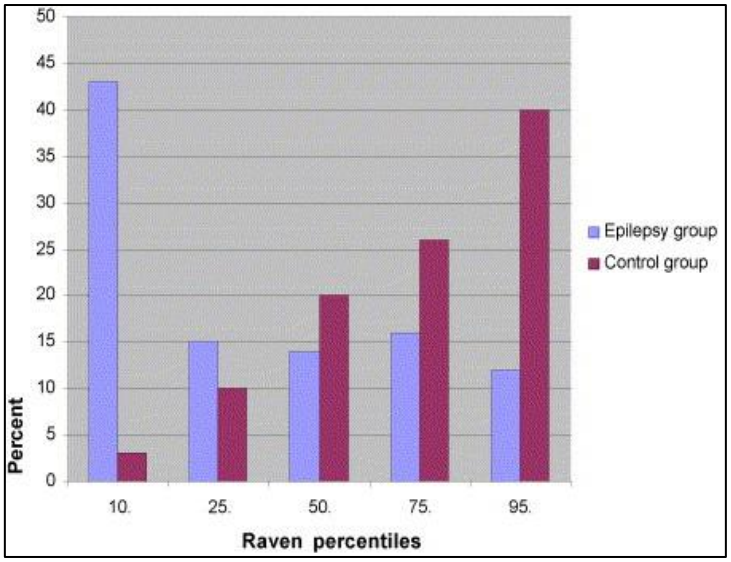


Figure 1: Raven matrices scores (non-verbal intelligence) between Norwegian children with epilepsy and normal healthy controls.This figure demonstrates the variation in cognitive profiles in children with epilepsy. Each bar is a percent of children which achieved a specific percentile. Y axis: Percent of children, X axis: Percentile scores in Ravens Matrices, 50th percentile average. Extract from Høie et al. (2005).

Why these normal non-verbal intelligence scores exist is unclear; it may relate to problems in experimental methods such as selection bias or type 1 errors. Clinically, it may be due to different epilepsy syndromes in the cohort, different aetiologies producing the phenotype or the ability of the child's brain and the environments they live in which mitigate the effect of the epileptogenic process on cognition. Nevertheless, the example above suggests that despite an association between epileptogenesis and cognitive impairment, they are not closely overlapped. Furthermore, it is unclear whether cognitive deficits are a part of or a by-product of epileptogenesis. In symptomatic epilepsies, it would be plausible to conclude that a large proportion of cognitive problems are secondary to the insult or lesion, whereas in idiopathic epilepsies this is far from certain. Despite this, there is evidence of adult patients with untreated epilepsy exhibiting cognitive problems at the onset epilepsy regardless of the aetiology (Witt and Helmstaedter, 2012). Also, there is evidence to suggest that in animal models of generalised epilepsies, there is a manifestation of manifest behavioural phenotypes before the generation of seizures (Jones *et al.*, 2008). These findings point towards the disruption of cognition before the development of seizures. Cognitive problems can also be a guide to identifying potential regions of brain dysfunction.

Cognitive deficits can sometimes provide useful clues in identifying ictogenic regions. In recognising deficits in intellectual functioning in symptomatic epilepsies, the features of the deficit can be used to lateralise or localise lesions (Helmstaedter 2004). However, to identify a potential site of dysfunction requires the examination of different cognitive abilities which include tests of IQ, attention, non-verbal skills, executive functions and memory, somato-sensory and motor function (Jones-Gotman *et al.*, 2010). A battery of tests allows for the dissection the patient's strengths and weaknesses and the identification of where in the brain could be the source of their cognitive problems. To a certain extent, the same can be done in idiopathic epilepsies, though this is not routinely performed. Nevertheless, using the same strategy as a neuropsychologist, the cognitive literature on idiopathic epilepsies can be used to extract the core cognitive problems and identify potential cortical substrates. The following section details the cognitive deficits identified in the children with idiopathic epilepsies.

2.2 Cognition in idiopathic epilepsies

The idiopathic epilepsies of childhood (IEC) consist of a diverse mix of focal and generalised epilepsies, which are age limited. In addition, like in RE, there is a prevalence of a variety of neuropsychological deficits. This section will present a brief overview of findings for intelligence quotient (IQ), academic performance, speech, language and literacy, visuo-spatial skills, attention and memory in the early and late childhood epilepsies with occipital paroxysms (CEOPs) (Panayiotopoulos syndrome (PS), Gastaut type occipital epilepsy) and childhood absence epilepsy (CAE).

2.2.1 Intelligence quotient

Even though there are statistically significant differences demonstrating lower intelligence quotient (IQ) in children with IEC, the majority will have an average or above IQ. The highest-quality studies included (Germanò *et al.*, 2005; Caplan *et al.*, 2008; Lopes *et al.*, 2013). In a controlled study of 90 children with IEC, Lopes *et al*. found there was a small significant difference (p=0.024) with healthy controls (Lopes *et al.*, 2013). In a controlled study of 22 children with CEOPs, they found that despite a low average full-scale IQ (91.7 ± 8.9) there was a significant difference with controls (103.5±9.9, p=0.005) (Germanò *et al.*, 2005). Similarly, a large controlled study investigated 69 children with CAE, they found full-scale IQ to be normal (101±15.61), but when compared to the control group (111±13.22), there was a significant difference (p=0.0001) (Caplan *et al.*, 2008). The use of 30 controls may have limited the Lopes study. However, the Germano and Caplan studies used similar or greater control group sizes with Caplan utilising age and gender matching. Weaker studies were uncontrolled, and these found normal IQ scores in IECs (Sturniolo and Galletti, 1994; Piccinelli *et al.*, 2010). Overall, despite a low average to average full-scale IQ scores deficits can be identified when compared to a control group. (Table 1). These deficits appear to translate into problems with academic performance, which occurs in around 50-60 % of children with IEC (Sturniolo and Galletti 1994, Bhise et al. 2010).

Study	N	Syndrome	Controls	Measures	IQ	Significant difference with controls
Lopes *et al.*, (2013)	90	Mixed	Yes	WISC III	Normal	Yes
Caplan *et al.*, (2008)	69	CAE	Yes	WISC-R (between 1994 to 1998) WISC III (between 1999 to 2005)	Normal	Yes
Piccinelli *et al.*, (2010)	43	Mixed	No	WISC-R	Normal	N/A
Sturniolo and Galletti, (1994)	41	Mixed	No	WISC	Normal	N/A
Germanò *et al.*, (2005)	22	CEOPs	Yes	WISC-R	Normal	Yes

Table 1: Summary of studies measuring IQ in children with IECs. Studies in order of sample size, large to small. WISC: Weschler Scale of Intelligence. WISC-R: Weschler Scale of Intelligence-Revised. IQ: Intelligence quotient. Red shading: Significant difference with controls. Blue shading: N/A.

2.2.2 **Speech and language**

The best quality studies would suggest that speech, and language problems exist in children with IECs. In a large controlled study of 69 children with CAE, around half had speech difficulties, with a significant difference in speech-language quotient (SLQ) when compared to controls (p=<.0001, Cohens d 0.6) (Caplan *et al.*, 2008). Their SLQ was measured with the Test of language development (TOLD) which consists of 9 subtests, none of the subtest results were reported. Similarly, Germano *et al.* found a significant difference (p=0.03, d 1.02) in language reception using the "Token Test". Moreover, both phonological (p=0.03, d 0.8) and semantic (p=0.02, d 0.99) verbal fluency were significantly different. Despite the evidence of mild receptive language dysfunction, a possible confound in this study was the control group which was not aged or sex-matched. Furthermore, fluency is more than a verbal skill as it reflects the integrity of executive and memory functions.

Smaller studies have not found any speech or language problems in CAE or CEOPs. A controlled study of 16 children with CAE found that there was no significance between groups in term of verbal IQ (Pavon*e et al.*, 2001). A retrospective study of 56 children with Panayiotopoulos syndrome found a discrepancy in their verbal (VIQ) versus performance IQ (PIQ) scores (>15 points difference) in 10.7% (Hirano et al. 2009). Both of these studies were weakened by their use of IQ measures to assess language and speech skills. The Hirano study, in particular, was lower in quality due to its retrospective methodology and lack of control group.

Despite a small collection of evidence, the best quality studies would suggest a prevalence of speech and language problems in the IECs. These appear to have large effect sizes in the CEOPs compared to CAEs. Subtle impairments in speech and language could be associated with impaired reading.

Study	N	Syndrome	Controls	Measures	Speech and language problems
Caplan *et al.* (2008)	69	CAE	Yes	TOLD	Yes
Hirano *et al.*, (2009)	56	PS	No	PIQ /VIQ discrepancy	~10%
Germanò *et al.*, (2005)	22	CEOPs	Yes	LTT	Yes
Pavone *et al.*, (2001)	16	CAE	Yes	VIQ	No

Table 2: Studies with speech and language assessments in children with IECs. Studies are in order of sample size; large to small. TOLD: Test of Language Development, PIQ: Performance IQ, VIQ: Verbal IQ, LTT: Language Token Test. Red shading: Evidence of problems in speech and language. Blue shading: No problems.

2.2.3 **Literacy**

Two small studies would suggest reading problems are prevalent in the IECs. The strongest study is Germano *et al*. found a significant difference in reading text error (p=0.005, d 0.88) against controls; they were also slower readers (p=0.009, d 1.36) (2005). It should be highlighted that the findings may have been influenced by a control group which on average were nine months older. Chaix *et al.* performed an uncontrolled study on 12 children with idiopathic generalised epilepsy group, which contained a mix of those with absences, GTCS (generalised tonic-clonic seizures) or both. They found that reading achievement was lower (but not significantly) than a RE group (Chaix et al. 2006). This impairment may have been apparent if compared to healthy controls, as there is evidence of reading impairment in RE (see section 2.3.2.2.1). Furthermore, two of the children in the IGE group had delayed language acquisition. Speech and language problems appear to be prevalent in both CAE and CEOPs these appear to translate into reading problems in both groups with large effect sizes in CEOPs (Table 3).

Study	N	Syndrome	Controls	Measures	Reading problems
Germanò *et al.* (2005)	22	CEOPs	Yes	Reading accuracy and speed test Sub-tests 4 (word list reading), 5 (non-word list reading), 10 (word dictation), 11 (non-word dictation) of Dyslexia and Dysorthographia Assessment Battery	Yes
Chai*x et al.* (2006)	12	CAE	No	Reading aloud meaningless text (l'Alouette) and a comprehension task (Le Printemps)	Yes, comparable to RE

Table 3: Studies which assessed reading in IECs. Studies in order of sample size, large to small. Red shading: Reading problems identified.

2.2.4 **Visuo-spatial skills**

There is a collection of good quality studies which suggest visuo-spatial problems in the children with CEOPs. In a controlled study of 20 children with CEOPs differences in the Benton visual retention test (p=0.005, d 1.38) and accuracy in selective visual attention (p=0.04, d 0.99) using the Developmental Test of Visual Perception (DTVP) were found (Germanò *et al*. 2005). A similar controlled study of 22 children found deficits in visual function in design memory (p=0.01, d 0.88) and visual learning (p=0.05, d 0.69) (Gülgönen *et al*. 2000). These findings were reproduced in a controlled study of 28 children with CEOPs and found a significant difference (p=<0.05) between groups in the Bender-Gestalt test (Polat *et al.*, 2012). There is difficulty in understanding what these results imply as the test used to assess visual function heavily relies on memory or manual drawing skills. It is a small collection of lower power data supporting visual dysfunction in CEOPSs; however, the data provides some strong evidence with moderate to large effect sizes. In CAE the date is lower in quality, yet there is also evidence of visuo-spatial deficits.

Two studies have found visuo-spatial problems in CAE both in the active and seizure remission phase. In a study of 16 children with CAE absences, using the WISC-R PIQ subtests, significantly impaired visuospatial skills (p=< 0.01) compared to controls were found, this was significantly (p=< 0.01) worse with early-onset (<4 years) (Pavone *et al.*, 2001)(2001). Furthermore, impairment has been recorded in seizure remission. In a study of 10 children with remitted CAE, they had significantly impaired performance IQ (p= 0.02) when compared to a RE group, which was attributed to impaired visuo-spatial function (Hommet et al. 2001). Both these studies relied on the performance IQ tests and thus are not specific for visuo-spatial cognition as they involve a mixture of memory, fluid intelligence and motor skills components.

There is good evidence of visual-spatial impairment in the CEOPs. In CAE, the data would suggest that perceived visuo-spatial deficits could likely be the result of deficiencies in memory, fluid intelligence and motor skills.

Study	N	Syndrome	Seizure remission	Controls	Measure	Visuo-spatial problems
Polat *et al.* (2012)	28	CEOPs	No	Yes	Bender-Gestalt test	Yes
Gülgönen *et al.* (2000)	22	CEOPs	No	Yes	Visual Learning tests and the Wide Range Assessment of Visual Motor Abilities	Yes
Germanò *et al.*. (2005)	20	CEOPs	No	Yes	Benton test and Developmental Test of Visual Perception	Yes
Pavone *et al.*, (2001)	16	CAE	No	Yes	PIQ	Possibly
Hommet *et al.* (2001)	10	CAE	Yes	No	PIQ	Possibly

Table 4: Visuo-spatial assessments in children with IECs. Studies in order of sample size, large to small. PIQ: Performance intelligence quotient. Red shading: Evidence of visuo-spatial problems.

2.2.5 **Motor function**

The best quality evidence would suggest that fine motor problems are prevalent in children with IECs. In a large controlled study, of 82 children with CAE, using tests of handwriting fluency and quality, they found dysgraphia in 20.7% of children with CAE and 7.8% of controls. They calculated a high risk for dysgraphia in children with CAE (OR: 3.49; 95% 1.2-8.8) (Guerrini *et al.*, 2015). Furthermore, within the dysgraphia subgroups, the children with CAE performed worse on a test of handwriting fluency (p=0.037) and in most trials of handwriting quality (p=0.02). In an uncontrolled cross-sectional study of 57 children with new-onset idiopathic epilepsy. To assess motor function, they used the grooved pegboard. Using the grooved pegboard, the group T score for the dominant hand was 39.2± 16.6, and non-dominant hand was 35.3±17.5 (Bhise *et al.*, 2010). Quality may have been affected by the lack of controls and prevalence of focal epilepsies in the Bhise *et al.* study. The largest studies of children with IECs would suggest that fine motor problems are prevalent similarly smaller sized studies found a mixed picture.

In the Germano *et al*. study, they used components of the Movement ABC test and found that static and dynamic balance and ball skills were not significantly different compared with controls, whereas manual dexterity was much lower (p=0.01, d 1.21) (Germanò et al., 2005). Conversely, Gülgönen *et al.* used drawing and a simple pegboard task from the Wide Range of Assessment of Visual and Motor Ability (WRAVMA) and found no significance. The Germano study, used the MABC which is specific for motor problems and the Gülgönen study used a non-specific measure which may have hidden motor problems. Similar fine motor results have been seen in CAE. In a controlled study of 12 children with CAE, the used a simple index finger tap test and found a significant difference (p=<0.05, d 1.04) between the CAE group and controls in the right hand but no difference in the left hand (Henkin et al., 2005). Medium-sized studies found mixed results, but large effect sizes were detectable. Overall, both large and medium-sized studies found evidence of motor dysfunction; in particular, these were in fine motor skills.

Study	N	Syndrome	Controls	Measure	Motor problems	Significant difference
Guerrini *et al.* (2015)	82	CAE	Yes	"Uno" and "LE" handwriting tasks	Yes	Yes
Bhise *et al.* (2010)	57	Mixed	No	Grooved pegboard	Yes	N/A
Germanò *et al.* (2005)	22	CEOPs	Yes	MABC	Yes	Yes
Gülgönen *et al.* (2000)	20	CEOPs	Yes	WRAVM	No	No
Henkin *et al.* (2005)	12	CAE	Yes	Finger tap test	Yes	Yes

Table 5: Studies with motor assessments in IECs. Studies in order of sample size, large to small. MABC: Movement ABC, WRAVM: Wide Range of Assessment of Visual and Motor Ability. Red shading, evidence of motor problems or significant difference with controls. Blue shading, no evidence.

2.2.6 **Attention**

The evidence would suggest attentional problems, are seen in a proportion of children with IECs (Table 6). In a large clinical trial investigating 393 children with drug-naive CAE, clinically significant attentional deficits were detected in 35% (Masur et al. 2013). Furthermore, in a controlled study 24 children with CAE, they found deficits in omission errors for divided attention (p=0.001, d .89), and reaction time in selective attention (p=0.004, d .68) (Cerminara et al. 2013). The quality of the evidence in the Masur study was marred by possible selection bias for those children with newly diagnosed epilepsy. In the Cerminara study, the other six measures of attention did not find a significant difference, and there was no correction of multiple comparisons, which could suggest a Type I error. Other confounds included the use of antiepileptic drug therapy in the Cerminara study, whereas, in the Masur study, children were excluded from the use of AEDs greater than seven days before testing.

In CEOPs, there is limited evidence of mixed quality. In a high-quality study by Germano, impaired attention (p=<0.05) was seen in 22 children with earlier onset CEOPS called Panayiotopoulos syndrome (PS) (Germanò et al. 2005). A lower quality study found still demonstrated a significant difference, Gülgönen *et al.* performed a controlled study on 21 children with mixed CEOPS they found evidence of impaired attention (p=<0.05) (Gülgönen et al. 2000). The quality difference between the studies was due to the task used. The Germano study used a cancellation task which is specific for visual attention, whereas Gülgönen used tests non-specific for attentional measures with a composite score. Overall, despite the lack of good evidence, the findings suggest that a proportion of children with IECs will have attentional problems.

Study	N	Syndrome	Controls	Measure	Attentional problems
Masur *et al.*, (2013)	393	CAE	No	Connors Continuous performance task	36%
Cerminara *et al.* (2013)	24	CAE	Yes	In-house continuous performance task	Possibly
Germanò *et al.* (2005)	22	PS	Yes	Bells test selective visual attention cancellation task	Yes
Gülgönen *et al.* (2000)	21	CEOPs	Yes	Derived from digit span, number-letter coding, symbol search, finger windows and picture completion	Possibly

Table 6: Assessments of attention in IECs. Studies ordered by sample size; large to small. Red shading: Evidence of attentional problems.

2.2.7 **Memory**

There is strong evidence to show that memory is impaired in CEOPs, whereas there is a lack of evidence of memory problems in CAE. In CEOPs, memory was found to be significantly affected in both the previously mentioned studies by Gulgonen *et al* and Germano *et al*. Germano found this to be the case using the Test of Memory and Learning (TOMAL) for both a verbal task; 'word selective reminding' (p=0.001, d 1.69) and visual task; 'memory for location' (p=0.002, d 1.47). Gulgonen used the WRAML and found 'sentence memory' (p=0.0154, d .78) and 'picture memory' (p=0.0149, d .79) to be significantly different. In CAE, the evidence for memory problems is lacking.

In CAE, the evidence for memory problems is lacking. D'Agati *et al*. found that there was no significant difference between 15 healthy controls and 15 children with CAE for verbal and visuospatial memory and verbal long-term memory (D'Agati et al. 2012). Pavone *et al.* saw similar results; they used the TOMAL in a controlled study of 16 patients with CAE. They found no difference in verbal memory; however, a significant difference was seen for non-verbal memory (p=< 0.05). However, the CAE group achieved an average score (49.9th percentile) compared to the controls (71st percentile), which may have influenced the results. All the children in studies were on anti-epileptic drug (AED) therapy, in particular, sodium valproate, despite this, there appear to be limited effects on memory in CAE. There is good evidence of memory deficits for both verbal and visual memory in CEOPs, whereas in CAE memory function appears to be unchanged, and their abilities are similar to healthy controls.

Study	N	Syndrome	Controls	Measure	Memory problems
Germanò *et al.*, (2005)	22	CEOPs	Yes	TOML	Yes
Gülgönen *et al*, (2000)	21	CEOPs	Yes	WRMAL	Yes
Pavone *et al.*, (2001)	16	CAE	Yes	TOML	No
D'Agati *et al.* (2012)	15	CAE	Yes	Digit span forward and backwards and Corsi block-tapping test	No

Table 7: Memory assessments in IECs. Studies ordered by sample size, large to small. WRAML: Wide Range Assessment of Learning and Memory TML: Test of memory and learning Red shading: Evidence of attentional problems. Blue shading: No evidence.

2.2.8 **Conclusions**

This review of the idiopathic epilepsies of childhood has revealed a predominance of low powered, small studies which have produced evidence of multi-domain cognitive dysfunction. This review has shown that cognitive problems are common in children with IECs. However, there is no standout cognitive deficit either within the syndromes or between them. Despite this apparent global disorder, some cognitive functions seem to be preserved, and this may be the reason why some of these children have IQ scores within the normal range.

Intelligent quotient scores are normal in groups of children with IECs however, when compared with healthy controls, there are significant differences in IQ. Significant differences in IQ compared to controls can be seen in both CAE and CEOPs and may be influenced by the verbal subtests. Average group full IQ scores and large standard deviations would suggest that there is varied intelligence in children with IECs. Why this is the case is unclear, but it is interesting to note that in the Germano study the children on average were seizure-free for 48 months and in the Caplan study, 71% had ten or more seizures in the year before the study. This additional information would suggest that deficits in IQ are unlikely to be related to active seizures, and other factors must be involved. Also, the deficits in IQ scores could be reflecting problems with working memory and speed of processing (Schatz *et al.*, 2000). Other neuropsychological assessments of children with IECs have found a variety of subtle deficits.

In children with IECs, there is a diverse collection of heterogeneous neuro-psychological impairments with similarities and differences between the groups. In particular, there are similarities across the IECs in problems with language and reading, speech and fine motor control and attention. Differences include memory and visuo-spatial abilities. These appear to be relatively well preserved in children with CAE, whereas in CEOPs these appear to be impaired. These findings would suggest different brain substrates behind the generation of these two epilepsies. Furthermore, the lack of neurological deficits would suggest that the primary cortices are spared, and these cognitive problems are arising from association cortices. However, to better understand the cognitive problems in IECs requires more studies with better research methods.

In the IEC literature, there is a limited number of studies which have issues with quality in both data collection and analysis. Why the evidence base is small is unclear, on the one hand, it could suggest that children with non-rolandic epilepsies have a lack of anecdotal evidence to warrant research or they appear not to require educational support. On the other, it is possible that the cognitive complications of IECs are overlooked because of their benign classification and their self-limiting nature. This review should dispel this belief and ask for a reconsideration of cognition in these children in active epilepsy and seizure remission.

Therefore, there is a need for large controlled cross-sectional or longitudinal studies to assess the impact of cognitive impairments in children with CAE and CEOPS. In addition, individual analyses or the calculation of expected odds ratios can help researchers better understand what proportion of children with IECs will have an issue with cognition and whether these children will have one or more cognitive deficits. This information may help further define these epilepsies and help clinicians and educator identify and provide adequate interventions for children with IECs.

The following section will explore the evidence of cognitive dysfunction in Rolandic epilepsy. It will demonstrate that there are more similarities than differences with the other IECs than what was previously understood. The literature in Rolandic epilepsy is far greater than the other IECs and thus will be explored in more detail and with a greater appreciation of specials issues such as the effects of inter-ictal spikes, seizures, seizure remission, familial cognitive problems and cognitive strengths.

2.3 Cognition in Rolandic Epilepsy

Cognitive problems in RE were first described by Beaumanoir and colleagues (1974). In a basic study, they found two patients with a left-sided spike focus achieved below normal performance on a digit recognition test and two with right-sided spikes, obtained lower-limit scores, on the Bender Visual-Motor Gestalt Test (Beaumanoir et al. 1974). Beaumanoir concluded that the spike side had a role to play in the type of cognitive deficit. At the other end of the evidence scale, a recent, large systematic review and meta-analysis analysed forty-two case-control studies using the Cattel-Horn-Carroll (CHC) model of intelligence. This study found large effect sizes for long-term storage and retrieval and small effects for visual processing (Wickens *et al.*, 2017). The finding of the meta-analysis was a novel and unexpected, but it is doubtful that this represents a unifying cognitive profile for RE. This section would suggest that both of these approaches to understanding cognition were flawed, and this review will demonstrate that the approach to cognition in RE needs to be far more nuanced.

Compared to the other IECs there is extensive literature detailing the cognitive deficits in RE, this section will explore the evidence for disorders in IQ, speech, language, literacy and hearing, visuo-spatial skills, attention, memory and motor and sensory function. Also, it will explore how these deficits change with time either by the impact of inter-ictal spikes, seizures or the developmental process. Furthermore, in selected modalities, neurophysiological evidence will be presented.

2.3.1 **Intelligence quotient**

Full-scale intelligence quotient (IQ) in RE is typically within normal limits despite this in some controlled studies; differences can be measured (Vannest et al. 2015). In a study of 30 children with RE, compared with children with a CAE, frontal lobe epilepsy and a control groups with 30 participants each analysis of variance (ANOVA) found a significant (p=0.024) (Lopes *et al.*, 2013). Another controlled study investigated 20 children with RE with the WISC III; they found despite normal IQs (lower in RE) there was a significant difference (p=0.017) (Völkl-Kernstock *et al.*, 2009). Conversely, Piccinelli *et al.* investigated with the WISC-R, 20 children with RE and 20 controls but found no significant difference (Piccinelli *et al.*, 2008) but this may have been a type 1 error. Similar, to the other IECs, most groups of children with RE will have normal IQs and can be significantly different when compared to controls. Investigating at the level of the individual reveals a complex picture.

It is quite difficult to obtain evidence of group variation in the literature; however, an example by Lopes *et al*. of 30 children with RE reveals a wide range of IQ scores (Lopes *et al.*, 2013). Seventy per cent of the children with RE had normal scores (≥ 90), and thirteen per cent of children with RE had borderline FSIQ scores of (70 to 79) which were comparable to the controls. Furthermore, there were a few children with RE with superior or very superior scores, which aged-matched healthy controls did not attain (Lopes et al. 2013). Potential confounds within this study were a mean age of six for seizure onset and the use of an old version of the Wechsler Intelligence Scale for Children III (WISC III). The newer versions of the WISC are far more nuanced in the measurement of FSIQ (Flanagan and Kaufman 2004). Despite the normality of IQ scores in this and other studies academic performance in children with RE is affected (Piccinelli *et al.*, 2008; Völkl-Kernstock *et al.*, 2009; Lopes *et al.*, 2013) which would suggest a specific deficit which full-scale IQ struggles to detect.

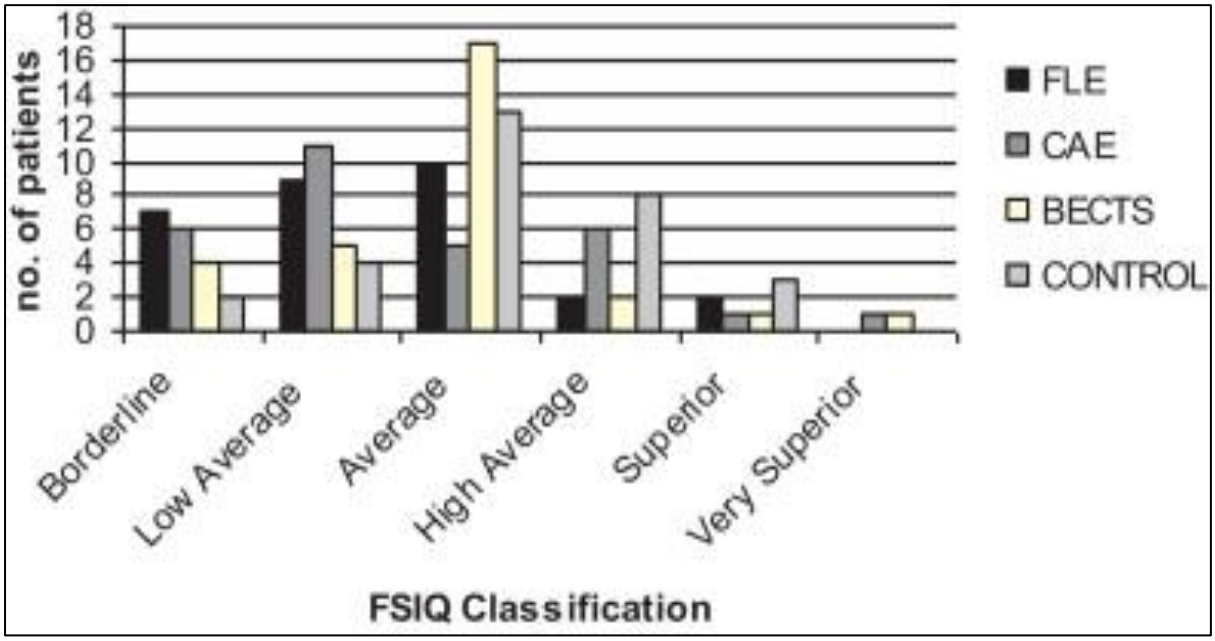


Figure 2: Full scale IQ in children with RE and other epilepsies compared to controls.

Children assessed with the Weschler Intelligence Scale for Children (WISC) in RE (BECTS), childhood absence epilepsy (CAE) and frontal lobe epilepsy (FLE) compared to healthy controls. This study demonstrates a large variation in full scale IQ in children with epilepsy which ranges from borderline to very superior. Extracted from Lopes et al. 2013.

2.3.2 **Speech, hearing, language, and literacy**

Language, literacy and speech impairments are the most commonly described problems in RE. A recent meta-analysis of twenty-two studies from 2000-2014 on language and literacy found a significant difference (<0.001) between children with RE and a comparison group (Smith et al. 2015). Specifically, this involved medium to large effect sizes in single word reading (d=0.71), expressive language (d= 0.75), receptive language (0.72) and to a lesser extent phonological processing (0.50). Homogeneity within these groups was measured by Cochran's Q; this was calculated as the weighted sum of squared differences between individual study effects and the pooled effect across studies. A large Q represents a heterogeneous sample (Cochran, 1954). The Q measure found single word reading (q = 8.22) and phonological processing problems (q=5.03) were similar across groups, whereas expressive (q=60.91) and receptive language (q=47.03) were heterogeneous (Smith et al. 2015). The meta-analysis found an increased incidence of speech and language problems in older children and those with reduced IQ. (Smith et al. 2015). Furthermore, linguistic difficulties may reveal themselves over time in older children (Smith et al. 2015). The following section will explore the evidence of impairments in speech, auditory function, language and literacy in RE and will suggest that there are many overlaps between the development of each of these cognitive functions.

The best quality evidence would suggest that speech sound disorders (SSD) a feature of developmental language disorders (DLD) can be seen in children with RE before or during epilepsy. In a cross-sectional study of 48 children with RE, Overliet *et al.* found a prevalence of a history of speech therapy (22.9%) and delayed the acquisition of speech (29.2%) (2011a). SSD can also be seen in active RE, in a cross-sectional study of 55 children with RE there was evidence of SSD in over a third (37%, odds ratio 2.54) (Clarke et al. 2007). Both of these studies relied on interviews or questionnaires, whereas others have physically assessed the children and found possible reasons for speech problems. A study of 20 children with RE found evidence of impaired lip and tongue movements (45%), difficulties in syllable articulation (30%) and impaired articulation of complex words (15%) (Staden et al. 1998). Rarely, a severe speech dyspraxia has been reported, and this has been associated with children with the autosomal dominant variant of rolandic epilepsy (Scheffer et al.

1995, Kugler et al. 2008). Furthermore, there are limited case reports demonstrating evidence of speech problems occurring after the onset of epilepsy. Berroya *et al.* reported on a normally developed five-year-old boy, who three months after seizure onset had difficulties in understanding and producing speech (2004).

In summary, the strongest evidence would suggest that SSD is prevalent in children with RE. Lower quality evidence would also suggest that SSD is prevalent, and this may be due to impairment mouth and tongue movements. Limited evidence of speech regression would imply that this phenomenon is rare or atypical. A delay in speech development in some children is due to hearing problems (Kennedy *et al.*, 2006; Rosenfeld *et al.*, 2016) however in children with RE this is unlikely to the case.

2.3.2.1 *Hearing, auditory function and phonological processing*

Difficulties in developing and maintaining speech production in RE has been hypothesised to be due to hearing, auditory function or phonological processing problems (Stoel-Gammon and Otomo 1986, Lundberg et al. 2005). The evidence, however, indicates that hearing is within normal limits. In a large retrospective study of 93 children with RE, Matos *et al.* found that all of the participants had normal audiometric hearing thresholds for pure tone and speech (Matos *et al.*, 2018). Similarly, Boatman *et al.* in a smaller controlled study of 7 children with RE found normal pure tone thresholds and tympanograms. The evidence would suggest that hearing is normal in RE. However, many children still complain of difficulties, and this may be due to cortical auditory processing problems.

Auditory and phonological processing problems appear to be prevalent in children with RE. Smith et al. in a cross-sectional controlled study investigated 40 children with RE. They found using the SCAN test for auditory processing disorders that in children with RE, there was an increased prevalence of an atypical left ear advantage in 25%. The atypical ear advantage was significantly higher than the 10% seen in the normative samples ($z= 3.2$; $p=0.001$) (Smith et al., 2017). Similar findings were reported in a controlled study of 24 children with RE. They used a dichotic listening task which utilised stop consonants and found an atypical left ear advantage (Bulgheroni et al. 2008). The evidence suggests that atypical auditory processing is common within children with RE; however, the studies presented have utilised tests which incorporate phonological content, and this may have confounded

the results. Some studies have used pure sounds to assess auditory function and deficits are apparent.

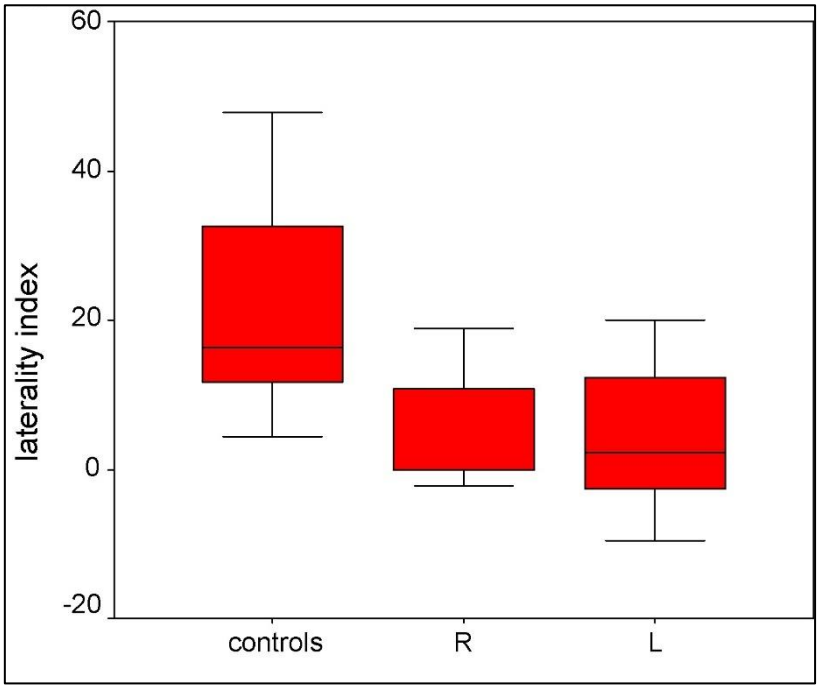


Figure 3: Laterality index in a dichotic listening test in healthy controls, and children with RE with right and left spikes. Laterality index: The higher the number the greater likelihood that the auditory information is processed by the right hemisphere. In this chart the controls demonstrate right hemisphere dominance for auditory processing whereas the children with RE, regardless of spike side appear to have an abnormal left hemisphere dominance. Extracted from Bulgheroni et al (2008).

The assessment of auditory processing of children with RE using non-phonological tests has produced mixed results which may have been influenced by the duration of the stimulus. Boatman *et al.* used a task where the children were asked to listen and count beeps. They found that there was no statistical difference between a group of children with RE and healthy controls (Boatman *et al.*, 2008) whereas different results were found for the gaps in noise (GIN) test. The GIN test is a task where participants have to identify millisecond gaps within white noise. In a controlled study 13 children with RE and a healthy control group, they found that there was a significant difference in both the gap detection threshold (p= <0.001, d 1.51) and percentage of correctly identified gaps (p= <0.001, d 1.38) (Amaral *et al.*, 2015). Interpretation of this study was complicated as the mean age of the children with RE was 11.6±1.8 years whereas the mean age of last seizure was 8.1±2.8 indicating that some of the participants may have been in seizure remission. These findings would suggest that

deficits in temporal auditory processing are apparent in children with RE, and this may persist into seizure remission. Overall, the evidence is limited, but it would suggest that children with RE have a deficit of auditory processing at a millisecond temporal resolution.

The neurophysiological data is mixed and reveals both normal and abnormal evoked potentials. Boatman *et al.* found in a controlled study of 7 children with RE that auditory brainstem response (ABR), were within normal limits for both latency and amplitude. Generation of the ABR is by the peripheral nervous system and subcortical structures. Similarly, there was no difference in the evoked N100 a potential, which is generated by the primary auditory cortex (Zouridakis, Simos and Papanicolaou, 1998) and is assumed to reflect the processing of frequency and loudness (Boatman *et al.*, 2008; Hickok, 2016). These findings would suggest that peripheral processing and initial cortical processing of auditory information is normal. Investigations of more complex stimuli which measure delayed cortical processing have produced mixed results.

The assessment of cortical processing of sounds in children with RE is normal for tones but becomes abnormal for speech sounds. Metz-Lutz and Filippini used a mismatch negativity (MMN) test in 23 children with RE with normal hearing with or without language impairment. They found that MMN was smaller but not significantly different in those with atypical RE with learning difficulties (Metz-Lutz and Filippini, 2006). Boatman *et al.* also found MMN to be not significant for tones, but there was an effect size for latency (d=0.52). However, distinct deficits were elicited when a speech stimulus was used, which consisted of a /ba/ standard and /da/ deviant. The MMN could not be recorded in 43%, and the remainder had a delayed cortical response (Boatman *et al.*, 2008). The Boatman study agreed with a previous study which identified in children with RE an absence of MMN to a similar speech sound paradigm (Liasis *et al.*, 2006). The evidence is limited, and it is important to note that these studies used different numbers of electrodes and possibly, different electrode locations. Nevertheless, these findings suggest that MMN can reveal a disruption in the cortical processing of speech sounds in children with RE. Atypical auditory processing can also be measured spatially.

Asymmetrical auditory evoked potentials with atypical distributions have been visualised in children with RE. In a controlled cross-sectional study of 12 children with RE, Liasis *et al*. found an asymmetry in the distribution of the P85-120 auditory evoked potentials which precede the N100 (Liasis *et al.*, 2006) (Figure 4). The distribution of the P85-120 potential in controls was over the mid-frontocentral

regions. Whereas in RE, it was contralateral to the reported inter-ictal spike side. Despite the small size, this data would suggest that the processing of auditory stimuli in children with RE is abnormal in the hemisphere where Rolandic spikes are generated.

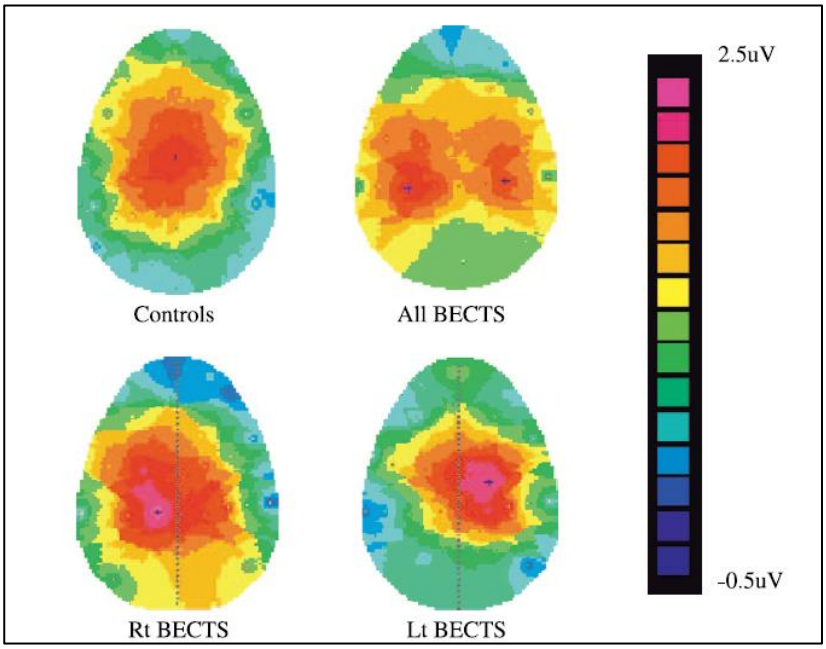


Figure 4: Voltage distribution maps comparing averaged P85-120 auditory evoked potentials in awake children with RE and controls. In children with RE there were significant differences in amplitude (p=0.014). None of these children had spikes in their awake recordings 20 minutes prior to the study. Note how the evoked potentials are contralateral to the regions of spike generation suggesting some kind of plastic or compensatory response to the cortical dysfunction (Liasis et al. 2006).

Overall, there is a scarcity of large studies investigating hearing, audiological and phonological function in children with RE. The studies have shown that the mechanics of hearing in children with RE and initial processing of auditory information is normal. Upstream auditory processing and phonological problems are prevalent within a proportion of this population, which creates the appearance of problems within hearing. Other deficits in auditory processing include atypical ear dominance and deficits of temporal auditory processing at the millisecond scale. Furthermore, there appears to be good, if rather limited evidence of the cortical processing of with speech sounds. These deficits in audiological and phonological function are not in isolation and appear to coincide with problems in language.

2.3.2.2 *Language*

There is good evidence for receptive, expressive language dysfunction in RE. In a systematic review, Teixeira and Santos analysed 18 studies and found that problems in semantic language skills were prevalent with additional evidence of morphosyntactic and phonological disorders (Teixeira and Santos, 2018). This study was in keeping with the previously mentioned systematic review by *Smith et al.* (Smith, Bajomo and Pal, 2015). These findings are also in keeping with the largest controlled studies. In a controlled study of 61 children with RE using the developmental neuropsychological battery (NEPSY), they found problems with verbal fluency (p=<0.001, d=1.24), speeded naming (p=0.001, d=0.8) and comprehension of instructions (p=<0.001, d 1.62). In particular, this was prevalent in children with early seizure onset *(p=0*.01) (2012) (Jurkevičienė et al. 2012). In keeping with this finding, another controlled study used the clinical evaluation of language fundamentals (CELF) core language scores in 25 children with RE and found core language scores (p=0.003, d 0.93). The sub-score analysis found deficits in semantic language both expressive (p=0.08) and receptive word (p=0.008) categories and concepts and following directions (p=0.01) when compared to CELF standard scores (Overvliet et al. 2013b). The difference in results may have been due to older children in the Overvliet study (11.33±1.92 years) compared to the Jurkevičienė group (9.5 ± 2.1 years) Nevertheless; there is good evidence to suggest that both expressive and receptive language

problems are prevalent in children with RE. In particular receptive language and comprehension are required for the understanding of written language.

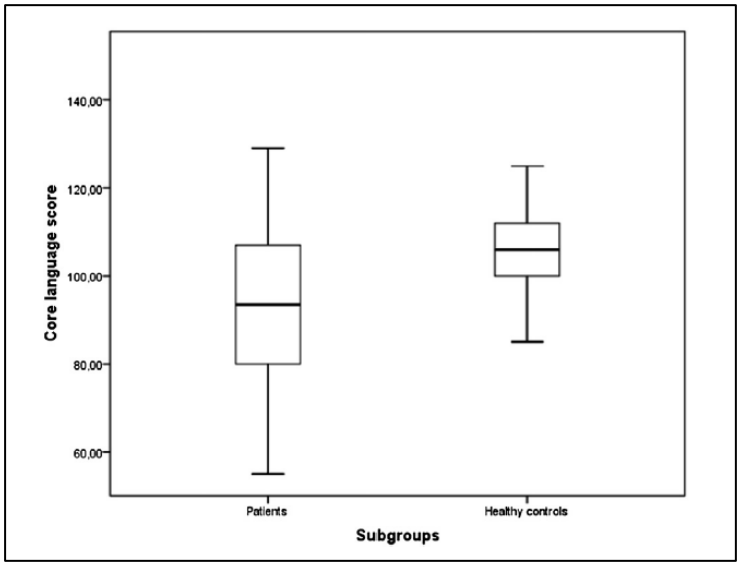


Figure 5: Core language scores in 25 patients with RE and 25 aged matched healthy controls. There is a significant difference between the group means (p=0.003) however, there is a wide range of language performance in the RE group (SD 18.4) compared to controls (SD 10.5). There is evidence of some children with RE performing better than controls. Extract from (Overvliet et al. 2013b)

2.3.2.3 *Literacy and grapheme processing*

In children with RE, there is a high prevalence of reading disability (RD) compared to the normal population. A prevalence for RD was demonstrated in the meta-analysis by Smith *et al.,* which found a moderate to large effect size for single-word reading (d 0.72). This finding is in keeping with a large observational study by Vega *et al.* of 108 children with RE. They found that the prevalence of reading disability was 42%, which was 3.5 more prevalent than the normal population (5-12%) (Shaywitz et al. 1990, Vega et al. 2015). In a controlled study by Oliveira *et al.*, 31 children with RE, 19.4% had evidence of RD (p=<0.001) (Oliveira et al. 2014). The Vega study relied on parental and school reports, which should be reliable, whereas, the Oliveira study included older children aged 14, increasing the likelihood of including individuals in seizure remission. It would appear that there is good evidence to suggest a large prevalence of reading disability in children with RE, which may improve as the child enters seizure remission. There is a possibility that the problems in reading may be due to visuospatial deficits.

2.3.3 **Visuo-spatial ability**

Neuropsychological testing has produced limited evidence of visuo-spatial problems in some children with RE. The best quality, controlled study used the “Differential Neuropsychological Test” (DNPT). Völkl-Kernstock *et al* found in 22 children with RE and impairment in spatial perception (p=0.001, d=2.03), spatial memory (p=0.001, d=2.01) and spatial orientation (p=0.001, d =99) (Völkl-Kernstock, Willinger and Feucht, 2006). Whereas an uncontrolled study found in 44 children with RE divided into typical (28) and atypical groups (16) that there was no significant difference for visuospatial ability (Metz-Lutz and Filippini, 2006). The Metz-Lutz study did not contain a control group, so it is difficult to ascertain if these children had measurable deficits compared to healthy children. Furthermore, the strength and robustness of the DNPT test used in Völkl-Kernstock study are questionable as the method was in house and unpublished. Overall there is contentious evidence of visual spatial dysfunction in children with RE. The neurophysiological evidence would agree with this position.

Visual evoked potential studies have shown that the early VEP components are normal, and late cognitive components are frequent. In an uncontrolled study of 11 children with RE Skrandies and Dralle, they found that early component of the flash VEP (N70, P100) were within normal limits however a late N200 was evoked in 5 children without a mismatch paradigm (Skrandies and Dralle 2004). The later N200 may be abnormal; however; it can be seen in 13% of healthy children and may be variant. The interpretation of this study was hindered by a lack of supporting neuropsychological data. The visual-evoked potential data is poor. Nevertheless, it would suggest normal primary visual processing subsequent cortical processing problems are unclear. In summary, there is a collection of low-quality evidence which would suggest no evidence for visuo-spatial processing problems in children with RE.

2.3.4 **Memory, attention and executive function**

2.3.4.1 *Memory*

In children with RE, there are deficits in memory, in particular, verbal memory. A systematic review found evidence of deficits in both verbal and visual-spatial short and long-term memory (STM. LTM) (Verrotti et al. 2014). Verbal STM deficits seemed to be the most prevalent. However, any further interpretation of this study was limited by the lack of meta-analysis. Despite this, the largest controlled study investigated 42 children with RE with the Wide Range Assessment of Memory and Learning (WRAML) and found deficits in 10 of the 12 subtests for memory (p=≤0.001) with a particular emphasis for problems in the encoding of verbal or visual memories. Interestingly encoding problems have been reported in smaller controlled studies.

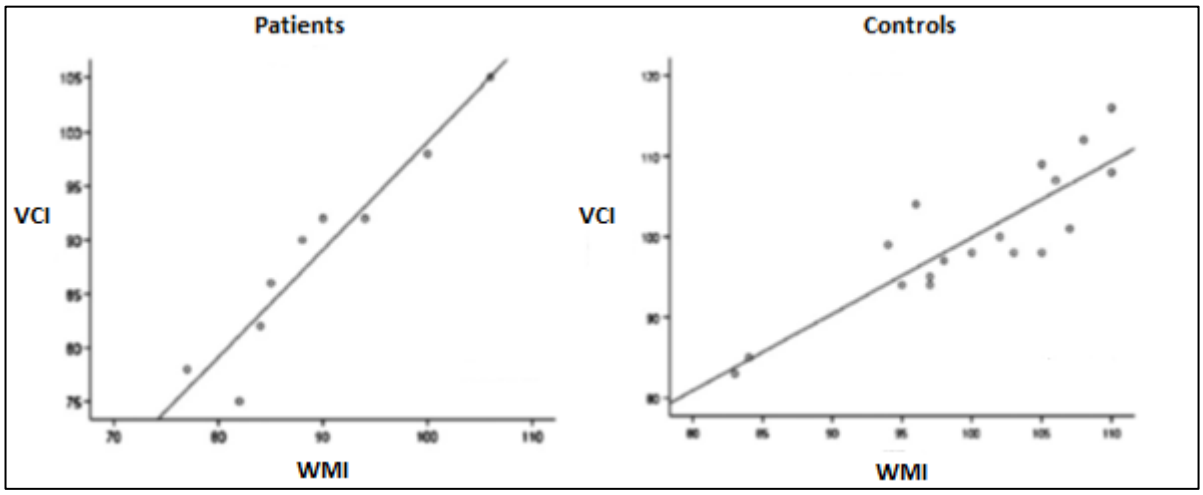


Figure 6: Verbal comprehension and working memory in children with RE and controls. Pearson correlation between verbal comprehension index (VCI, a measure of verbal acquired knowledge and reasoning) and working memory index (WMI, a measure of short-term memory). WMI in RE appears to lower compared to controls. There appears to be a positive correlation between VCI and WMI and this is to a lesser extent in controls. Extract from (Verrotti et al. 2013)

In a controlled study of 25 children with RE using the KET-KID a German psychological test of verbal and non-verbal abilities, they identified problems with auditory (p=0.003) and visual (p=0.016) memory (Danielsson and Petermann, 2009). Similarly, in a controlled study of nine patients with RE using the Weschler Intelligence Scale for Children-IV (WISC-IV), Verroti *et al* found a significant difference (p<0.008, d 1.22) in working memory index which correlated with the verbal comprehension

index (Verrotti *et al.*, 2014) (Figure 6). It is important to note that the Danielsson and Peterman was retrospective and used non-parametric statistical analysis implying a non-normal distribution in the sample. Furthermore, the WMI found a deficit, but the subsequent NEPSY-II analysis only found a memory deficit for lists and not for immediate or delayed memory for designs. Overall auditory and verbal encoding appears to be impaired, and this could lead to apparent long-term memory problems.

Long term memory can be affected in RE; this is predominantly verbal memory. Vago *et al.* found in a controlled study of 24 children with RE. They used the California Verbal Learning Test (CVLT) and found that with delayed recall of 20 minutes the number of recalled words was significantly reduced (p=0.017, d.76) in younger children (<10 years). In older children with RE (>10 years), there was no difference (d .05) (Vago et al. 2008). Similarly, in a controlled study of 17 children with RE using the Rey Auditory-Verbal Learning Test (RAVLT), delayed recall was difficult at 5 minutes (p= <0.01, d 1.15) and 30 minutes (p =<0.01, d 1.25). This deficit was not apparent for a visuo-spatial memory, in the children with RE, their aptitude for the complex figure of Rey was comparable to controls demonstrating strength in visuo-spatial memory (Croona *et al.*, 1999). The majority of younger children in the Vago study had multi-focal Rolandic spikes which may have influenced the results. Whereas the Croona study may have been influenced by an unrepresentative female predominance (around 59%) and five of the children had nonspecific MRI abnormalities.

In summary, there is a fair amount of good evidence indicating deficits in verbal STM and LTM in children with RE. Also, the evidence suggests an apparent sparing of visuo-spatial memory. Nevertheless, a meta-analysis of published results and a call for grey literature would improve the understanding of this deficit. It appears that other executive functions, such as attention appear to be dysfunctional in children with RE.

2.3.4.2 *Attention and executive function*

There is strong evidence for attentional deficits in children with RE. The strongest evidence was a systematic review of 21 studies on attention in RE (Kavros *et al.*, 2008). Using the Posner model, they subdivided attention into alerting, orientating and executive networks. They found evidence that each category showed signs of dysfunction. These deficits would be apparent when Rolandic spikes were on the EEG and would remit when the EEG normalised (Kavros *et al.*, 2008). Failings of the review were the inclusion of tasks such as the Stroop and dichotic listening which are known to be influenced by verbal ability and auditory functions respectively (Homack and Riccio, 2004; Musiek and Chermak, 2015). Furthermore, there is a need for meta-analysis. Overall, the finding of the review would suggest that attentional problems are prevalent and have a global impact on cognition in children with RE. Other executive functions appear to have limited dysfunction.

Deficits in executive function are limited in children with RE. The largest controlled study involved 25 children with RE. They assessed children using the Wisconsin card sorting task (WCST), a trail making test (TMT) and a verbal fluency task (FAS). The children with RE performed worse than controls but not significantly(Neri *et al.*, 2012). Another controlled study of 15 children with RE tested executive function using the five-point test, a structured figural fluency task and an alpha span tests. The alpha span task relied on a list of words which were presented in blocks with increasing length; these blocks had to be recalled in alphabetical order. There was a significant difference in the number of five points correct ($p=0.003$) and the number of errors ($p=0.008$), the alpha span was not significantly different (Filippini *et al.*, 2016). In the Filippini study, the patients had new-onset RE and were drug naïve, which raises questions about the long-term outcome of these children and whether they will keep their diagnostic label. In the Neri study, a proportion of participants were in seizure remission, and the structured figural fluency task relied heavily on drawing, a motor skill. Overall there is good evidence of impaired attention across many domains whereas there is limited evidence of impairment executive function. As demonstrated in the five-point test, there is evidence for a deficit in motor abilities.

2.3.5 **Motor function**

There is a large amount of good quality data to suggest that a proportion of children with RE have problems with motor function in particular fine motor function. In a large controlled study, 44 children with RE were evaluated using the Purdue pegboard test. After controlling for intelligence, there was a significant different in dominant hand (p=0.027, d 0.61), both hands (p=0.045, d=0.69) and assembly (p=0.046, d=0.64) (Ayaz *et al.*, 2013). Interestingly, the non-dominant hand was not significant (p=0.055, d=0.54) but had a moderate effect size. This study may have been biased by selecting children over eight years of age and the lack of correction for multiple comparisons. Nonetheless, these findings would suggest that children with RE have evidence of fine motor deficits, which may be part of the diagnosis for developmental coordination disorder (DCD).

A medium-sized uncontrolled study investigated DCD in 21 children with RE found that nearly half (47.6%) of the children had some difficulties in one or more areas of motor functioning (Kirby *et al.*, 2017). Eight of these children (38%) fulfilled an indication for DCD using the DCD questionnaire (DCDQ'07), five (23.8%) scored below ≤ the 5th percentile of the Movement ABC task and three (14%) scored below the 5th percentile in the detailed assessment of the speed of handwriting task (DASH) (Kirby *et al.*, 2017). None of these children had a DCD diagnosis before testing. Despite the small study and no control group, these findings would suggest that the potential for DCD is prevalent in children with RE. The previous studies demonstrate that motor problems for children with RE are prevalent but none specific with a proportion exhibiting severe problems. It is unclear whether these motor problems in RE are preceded by developmental delay.

There are a few studies which suggest that children with RE have a delay in learning to walk. In an uncontrolled study of 48 children with RE, they found "problems in motor development" in 25%, but they did not provide data on how delayed these children were (Overvlie*t et al.*, 2011). Another uncontrolled study by Gunduz et al. investigated 20 children with RE and found a delay to walk in 20% (Gündüz et al. 1999). Unfortunately, this evidence was confounded as 25% of the children had consanguineous parents moreover, "a delay" was not clearly defined. Two uncontrolled studies provide evidence that a delay in walking could occur in a small proportion of children with RE.

Therefore there is a need to perform controlled studies in this area. Similarly, another motor function, speech can be delayed in children with RE.

In the study above by Overvliet *et al.* there was evidence of a delay to language development in 29.2% of the cohort. Another, controlled study of 25 children with RE found that 40% of the RE group had speech therapy compared to 24% in the control group. This finding was suggestive of aberrant speech development. Unfortunately, in both of these studies, the delay to speech is not defined, and it is unclear whether speech therapy intervention was due to motor or language issues. Nevertheless, a delay in the development of speech can be a sign of neurological dysfunction in children with RE this can present as mild neurological motor signs in the mouth muscles and speech dyspraxias.

Mild localising neurological signs can be seen in children with RE, predominantly in the oral muscles and tongue. In a controlled study of 20 children with RE using an assessment of tongue movement, they found a significant difference ($p=<0.05$) with controls. Furthermore, the children had problems with articulation ($p=<0.01$) (Lundberg *et al.*, 2005). Another study of 20 children with RE, found that some had impaired lip and tongue movements (45%) and difficulties in articulating a sequence of syllables (30%). One of the participants had severe speech dyspraxia, which was apparent in everyday speech (Staden *et al.*, 1998). Acoustic speech analysis on 18 children with RE, also found 72.2% of the children had evidence for the impairment of the coordination of articulatory movements which result in speech dyspraxia (Pal *et al.*, 2010). It is important to note that the Lundberg studied children who may have been in seizure remission (mean 15 months seizure free) and Staden studied children with very active epilepsy (only three had seizure freedom >1 year). Further, the studies described are small and most uncontrolled, which demands further exploration with large controlled studies and systematic review. In summary, there is evidence to suggest that motor problems in children with RE are prominent and prevalent; these issues may be related to sensory dysfunction.

2.3.6 **Peripheral sensory function**

There is evidence of abnormal sensory processing in children with RE, but the data is limited. Tactile and electrical sensory stimulation has been used to elicit extremely high amplitude cortical evoked potentials, which could be interpreted as Rolandic spikes. Manganotti *et al.* studied six patients with RE, using a triggered tendon hammer and digital electrical stimulation they evoked EEG potentials with a morphology similar to a Rolandic spike contralateral to the side of stimulation (Manganotti *et al.*, 1998). A slow stimulation rate of 1 Hz repeatedly generated a single high amplitude somato-sensory evoked potential (SSEP) whereas increasing the rate to 10 Hz abolished the phenomena. This study was performed in a small cohort of children who have spikes in their awake EEG, which some physicians may describe as atypical. Overall, there is a distinct lack of evidence exploring peripheral sensory function in children with RE.

2.3.7 **Summary**

The previous sections have attempted to provide a comprehensive overview of cognition in children with RE. The prominent cognitive deficits appear to be in speech, language and reading, motor skills, attention and verbal short- and long-term memory. The following section, on special issues, will explore the effect of inter-ictal spikes, seizures, seizure remission, familial cognitive problems. Furthermore, it will investigate the overlaps between cognitive deficits and the possibility for specific cognitive strengths.

2.4 The effects of spikes and seizures on cognition

2.4.1.1 *Inter-ictal EEG features and cognition*

Despite the theoretical literature on spikes influencing cognition in epilepsy, there is little evidence of this occurring in children with RE. It is hypothesised that spikes impact cognition in two ways, one is immediate and the other occurs on a medium to long term scale. In the immediate, focal inhibition generated after the spike may be responsible for a phenomenon called transient cognitive impairment (TCI) (Shewmon and Erwin 1988, Binnie 1993, Aldenkamp and Arends 2004, Nicolai and Kasteleijn-Nolst Trenité 2011). In the medium to long term, spikes are suspected of inducing long term molecular changes, cell death or sprouting of axonal projections (Lenck-Santini and Scott 2015). The following section will demonstrate that the evidence is weak for spike mediated cognitive dysfunction both in short to medium term.

2.4.1.2 *Spikes, transient cognitive impairment and attention.*

In the short term, the evidence for transient cognitive impairment is limited and weak. Aarts *et al.* define TCI as “functional deficits demonstrable by suitable testing, during spike or spike-wave discharges, which are subclinical in the operational sense” (Aarts *et al.*, 1984). Binnie states that TCI is seen in fifty per cent of children with epilepsy (2003); however, this is unlikely to be the case in children with RE. In a study of 10 children with RE, they found six out of the 7 with participants with typical features demonstrated “an increased error rate in trials accompanied by EEG discharge” (Binnie, 1993). Furthermore, in a study of 17 children with RE with Rolandic spikes, while awake, two of the participants had evidence for TCI (Fonseca, *et al.*, 2007). However, TCI was characterised as “a statistically significant greater proportion of errors during RS-containing than during RS-free periods”. Both of the studies would appear not to be in keeping with the original definition of TCI. Furthermore, Fonseca *et al.* found that the “TCI” did not translate into poor academic performance.

There are two small studies which focus on TCI in children with RE. Overall, the evidence is weak for TCI it would suggest that this is an unlikely and atypical phenomenon which are related to diurnal spikes but not time locked. Despite the lack of a time-lock, there is evidence to suggest impaired attention.

2.4.1.3 *Diurnal spikes and cognition*

Subject	Sessions	Spike rate (per minute)	Spike side	Asynchronous focus
4	1-2	**2.3-0**	**Lt-N/A**	N-N
	2-3	**0-4.8**	**N/A-R**	N-N
5	1-2	**0.8-3.8**	B-B	Y-Y
	2-3	**3.8-0.8**	B-B	**Y-N**
6	1-2	0.7-0.2	**B-R**	N-N
	2-3	0.2-0.8	**R-L**	N-N

Table 8: Changes in spikes and EEG features over serial awake recordings. Recordings were conducted 4-5 weeks apart. Values in bold indicate significant change. Lt=left, Rt= right, B=bilateral, N=no and Y=yes. This table demonstrates that spikes in individuals with RE frequently vary in their appearances, hemisphere and distribution. Extracted and adapted from Ewen et al. (2011) participants with three successive EEGs included.

The evidence for altered cognition concerning diurnal spikes is weak. A longitudinal study of six children with RE performed neuropsychological testing after EEG recordings. They found significant changes between sessions in visual-spatial organisation (±2.12), attention (±1.72) and verbal memory (±1.52) (p=<0.05) (Ewen *et al.*, 2011). The EEG data demonstrated diurnal spikes which changed in density between successive recordings (. Unfortunately, due to the low sample size, they were not able to calculate the correlation between changes in spike density and cognition, but the study concluded that visually there were trends. The Ewen study was low powered and included patients with EEG slowing, which some physicians would see as evidence against idiopathic epilepsy (Britton

et al., 2016). Nevertheless, they found that fluctuations in awake EEG spikes did not correlate with cognitive deficits; there is a suspicion that the location of diurnal spikes may influence cognition.

Despite a large number of studies on this question, there is no substantial evidence for diurnal spike location influencing cognitive deficits in children with RE. A few studies have found that right-sided spikes while awake have been associated with deficits in visuo-spatial skills and memory and left-sided spikes with verbal and language deficits (Wolff et al. 2005, Bedoin et al. 2006, Riva et al. 2007). However, the majority of studies which collectively have examined a greater number of children with RE, have shown no relationship (Liasis et al. 2006, Piccinelli et al. 2008, Goldberg-Stern et al. 2010, Jurkevičienė et al. 2012). Furthermore, those who confirmed an association relied on awake EEG recordings performed 2-3 months before testing. This brief overview of spike lateralisation and associated cognitive deficits demonstrates that the evidence is weak, and to improve interpretation; there is a need for systematic review and meta-analysis. It may be that spike morphological features or non-spike grapho-elements influence cognition.

2.4.1.4 *EEG grapho-element morphology*

The morphological features of spikes and other electrographic grapho-elements may influence cognition. A study of atypical RE phenotypes found that there was a significant correlation with multiple asynchronous foci, prolonged clusters and an abundance of spikes both while awake and in sleep (Massa et al. 2001). Furthermore, if the spikes had associated clinical symptoms, such as positive or negative myoclonus, the child would have poorer cognition (Massa et al. 2001). Moreover, slow waves and generalised slow-wave discharges with low amplitude spikes were associated with a cognitive deficit (Massa et al. 2001). It is thought that these features are a representation of cortical immaturity although they may not be apparent in serial EEG recordings (Eeg-Olofsson et al. 1971, Massa et al. 2001, Ewen et al. 2011). Massa provides convincing evidence, but some of her findings do not stand up to repeat studies.

In a retrospective study, Nicolai *et al.* investigated 28 children with RE. They found that atypical EEG criteria did not discriminate absolutely between a benign or complicated evolution. Nevertheless,

significant correlations were found between awake intermittent slow-wave foci (p=0.042), in the first hour of sleep, high spike densities (p=<0.0001) and multiple asynchronous bilateral spike-wave foci (p=0.036) and a high density of spike across the whole night's sleep (p=<0.0001) with an deleterious effect on cognition (Nicolai et al. 2007). This study may have been influenced by 21.4% of the individual incurring a complicated evolution of their epilepsy and the use of 21 measurements with no correction for multiple comparisons. Overall, there is weak evidence for atypical features being associated with abnormal cognition, to improve understanding prospective replication studies are required. The Nicholai study suggests that nocturnal spikes have an impact on cognition, and a large proportion of the literature supports this position.

2.4.1.5 *Nocturnal spikes*

The evidence for nocturnal spikes influencing cognition is much stronger, but the nature of the association is unclear, and the number of studies is low. A large study of 42 children with RE found correlations between spike frequency and CELF-3 semantic word associations (p = 0.003) and seconds in trains of spikes correlated with the WISC-3 digit span (p = 0.043), and WISC-3 freedom from distractibility (p = 0.048) (Northcott *et al.*, 2005). Another study retrospectively investigated 26 children with RE using the Tempo Test Woorden and Zinnen. They found a correlation between increased amounts of nocturnal spikes and decreased performance in reading sentences (p = 0.008) (Ebus *et al.*, 2011). However, single word wording revealed no differences. Finally, Baglietto *et al.* studied a smaller cohort of nine children and only reported spike densities and not how they related to cognitive measures; this would suggest that no relationship was found (Baglietto *et al.*, 2001). Despite these findings supporting an association between nocturnal spikes and cognition, there are methodological concerns.

Most studies which have investigated the effect of nocturnal spikes on cognition have poor study design and do not measure concomitantly. Ebus and Northcott collected neuropsychological data, two weeks and 15 months respectively after EEG recording. Baglietto did collect data concomitantly but did not measure language or reading. To improve the methodology requires the use of longitudinal

studies with several time-points with a comprehensive battery of neuropsychological tests. Overall, despite poor methodology, the literature would moderately support an association between nocturnal spikes and cognitive problems. It may be that a static nocturnal spike generator could have detrimental effects on cognitive abilities.

Static nocturnal inter-ictal spikes may be detrimental to cognition in children with RE. In a controlled study of 20 patients with RE, in those with a diagnosed specific learning disability (SPLD's), they had specific inter-ictal EEG abnormalities, which appeared in serial sleep EEGs over the same region for longer than a year (Piccinelli et al. 2008). Moreover, these static nocturnal spikes were seen during 50-85% of the sleep period. The Piccinelli study included children aged 7 and 12 years with an epilepsy onset between 2-11 years, which suggest in some participants a long duration of epilepsy; indeed, 12 of the children were seizure-free. Nevertheless, Piccinelli's study would suggest that non-dynamic spikes during sleep, which persist for long periods could be detrimental to cognition. Nevertheless, this is one study, and it requires further investigation. The evidence points towards a role in nocturnal spikes in cognitive dysfunction; the process may be by disrupting sleep processes.

The evidence would suggest that despite children with RE having inter-ictal spikes, there is weak evidence for poor sleep quality. Bruni *et al.* compared ten children with RE and ten controls. He found a reduction in total sleep time (TST) (p.046 d 1.06), sleep efficiency (p .035 d 1.10), increase in rapid eye movement (REM) sleep latency (p.008 d 1.47), and cyclic alternating pattern (CAP) rate (p .012, d 1.36) (Bruni et al. 2010). Despite this, there appeared to be no relationship between spike activity and CAP components. This study was hindered by its small sample size, but the effects seen are quite large. Other studies have found no effect but have used similarly used small cohorts (Dalla Bernardina et al. 1975, Clemens and Majoros 1987, Nobili et al. 1999). Overall, sleep is affected in children with RE, but the evidence for spikes having this effect on sleep is weak.

Spikes are prevalent and dominant on the EEG in children with RE, yet the data supporting their influence on cognition is limited, and the findings are weak. The evidence for TCI or diurnal spikes influencing cognition in children is poor. Similarly, the data on atypical spikes and grapho-element features is not reproducible and requires further investigation. The case for nocturnal spikes, rather than diurnal spikes influencing cognition has greater strength, but these studies need to be repeated with better, robust designs. Indeed, if an association was found using longitudinal measures, the

direction of association should also be explored. Overall, this review of inter-ictal features would recommend a cautious approach to the association between spikes and cognitive problems. In the following section, the effect of ictal and peri-ictal events will be discussed.

2.4.1.6 *Ictal events and their effects on cognition*

Seizures do not appear to correlate with disorganised cognition. A study investigated 36 children with RE using the WISC-R, Kaufman assessment battery for children (K-ABC), Rey-Osterrieth complex figure and the Rey Auditory learning test. They found that there was no relationship between seizure frequency and poor scores (Goldberg-Stern *et al.*, 2010). Counterintuitively increased seizures were associated with significantly improved scores for better vocabulary in the WISC and verbal learning (Goldberg-Stern *et al.*, 2010). In contrast, Papavisiliou *et al.* performed a controlled longitudinal study on 32 children with RE investigating reading skills. They found a reduction in seizure frequencies in patients with no or mild written language problems compared to those with poor performance. (Papavasiliou et al. 2005). The main confound for these studies is whether all seizures have been successfully detected, which is a particular problem in the paediatric population (Sidenvall et al. 1993). There is limited evidence of seizure related cognitive problems however, there may be an association between seizure semiology and cognitive profile.

Some research groups have suspected that the seizure semiologies in RE are linked to different cognitive profiles. Two hundred divided two hundred children with RE into various seizure semiologies and found a significant link between verbal learning problems and those children with partial seizures only (p=<0.01) (2006) (Giordani *et al.*, 2006). Verrotti *et al*. monitored children with RE, 64 with typical and 8 with atypical seizures. His team found that 8% of those with typical seizures had speech delay compared to 46% in the atypical group (Verrotti et al. 2002). In concordance, a retrospective study of 50 children with RE identified atypical seizure characteristics which included leg jerking, lateral body torsion, uni-lateral body sensations, epigastric pain and ictal blindness. They found that these features positively correlated with impaired language function (p=0.021) (Vinayan et al. 2005). The Giordani despite having a large sample size had methodological problems, there was a bias for participants with frequent seizures, as they were assessed before an anti-epileptic drug trial and cases were defined as RE from RS on EEG and negative neuroimaging. In the Vinayan study, 20% had adverse peri-natal events such as pre-maturity, minimal birth asphyxia and neonatal jaundice. Finally, the Verotti study included a very small cohort with atypical seizures, which makes a comparison difficult. The literature provides poor methodologies and mixed evidence; as a result, frequent or atypical

seizures may be associated with poor cognition. Overall, further longitudinal research is required to see what the directionality of the association between seizures and cognition and what is the effect on those children with an atypical seizure classification over time. There are a few papers which would suggest an early age of onset is detrimental to cognition.

Age of onset could be an important impact on cognition, but the information is limited. It is widely quoted that young children of eight years or younger are more likely to have cognitive deficits (Piccinelli et al. 2008). However, in Piccinelli's controlled study of 20 children with RE, the age range was restricted (around eight to thirteen years), which is a small and neglects younger children. Conversely, a retrospective study, in 24 children with RE found that aged eight years or over were more likely to have deficits compared another group with a mean age of 5 (Filippini et al. 2013). Both of these studies were small, and Fillipini may have biased the results by recruiting children from a specialist child neurology hospital. The evidence is weak to support an association between early onset of seizures and poor cognition. However, it is necessary to keep in mind that first seizures may be subtle and unrecognised.

In summary, similar to inter-ictal abnormalities, there is a lack of strong evidence to suggest a relationship between seizures and cognition. There is still a large amount of longitudinal controlled studies to be done to improve the understanding of this topic. Nevertheless, an interesting strategy to look at cognition in children with RE is to perform neuropsychological tests during active epilepsy and repeat when their spikes and seizures remit, which is the focus of this thesis.

2.5 Longitudinal studies in RE and seizure remission

2.5.1 *Longitudinal changes in cognition*

Cross-sectional studies have found as a child with RE matures epileptiform features and seizures will disappear (Kim *et al.*, 2018) and their cognition will improve (Lerman and Kivity, 1975; Alessandro P *et al.*, 1990). Moreover, a systematic review of attention found in spike remission, there was limited evidence of attentional problems (Kavros *et al.*, 2008). Conversely, recent studies have found residual cognitive deficits. In a retrospective study of 33 children with RE they found that deficits persisted in those children with either a large density of inter-ictal spikes in NREM sleep, onset of RE at school age or were taking multiple AEDs (Filippini et al. 2013). These studies provide a mixed picture suggesting that cognition, in particular, attention improves with seizure remission; however, this view is mostly derived from mixed quality cross-sectional studies. Therefore, this section with predominantly focus on longitudinal studies of cognition in seizure remission and explore what effect time in remission, sample size and the typical/atypical dichotomy of RE has on interpretation.

Longitudinal studies demonstrate that cognition will improve over two years of seizure remission. A controlled longitudinal study of 24 children with RE showed a measurable increase in cognitive ability over two years in both the RE and control group; however, at time-point two, a RE group deficit remained. The deficits included a significant difference in tasks such as confrontation naming ($p=0.006$), immediate verbal memory($p=0.005$), the WISC-III coding task ($p=0.001$) and the grooved pegboard ($p=0.001$). Interestingly, a significant improvement ($p=0.001$) was seen in arithmetic skill measured by the Wide Range Achievement Test-3 (WRAT-3) (Garcia-Ramos et al. 2015). The majority of children (62.5%) were seizure-free or in seizure remission at the follow-up, which suggests that cognitive improvement can still occur despite having spikes and experiencing seizures. Another smaller study found the same phenomena.

A controlled study of 9 children with RE found a significant difference at baseline in verbal IQ, trail-making with letters, block tapping ($p=<0.01$), Stroop fluency ($p=0.001$) and naming, visuo-motor and spatial abilities ($p=0.0001$). In the two year follow-up, there was an improvement in cognition in visuo-

motor coordination and perceptual performance, non-verbal short-term memory, sustained attention and mental flexibility (Baglietto *et al.*, 2001). Despite this, some differences remained in trail making with numbers (p=0.01) and spatial abilities (p=0.001). An improvement was seen, despite the presence of RS in the sleep EEG recordings in 44% of participants at the follow-up. A limitation of this study was that raw neuropsychology scores were used for the majority of the analysis, and this may have hidden deficits. Furthermore, there was no correction for multiple comparisons. These studies provide evidence of improvement in cognition in the short term; however, it is unclear whether this improvement mitigates all of the cognitive issues. Longitudinal studies with a long delay in follow-up and a greater number of children in seizure remission show both improvements and deficits.

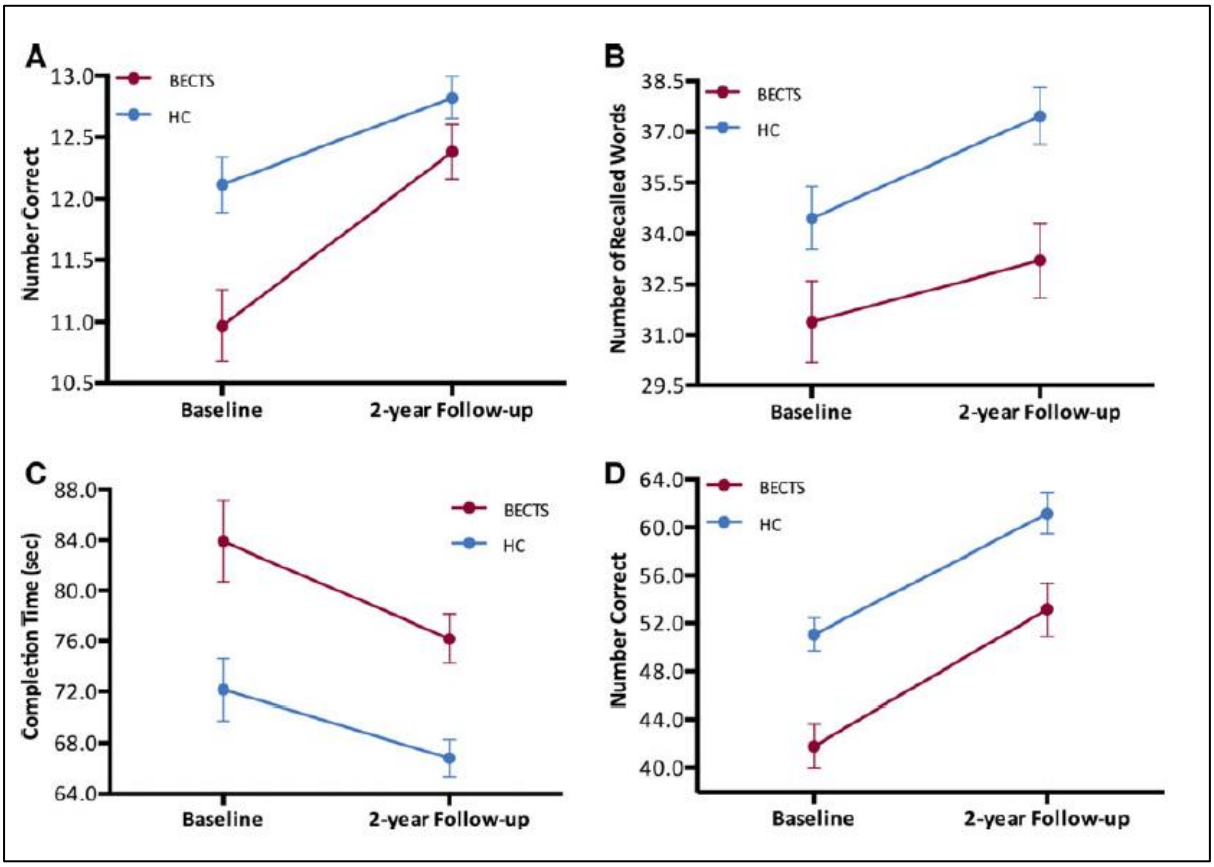


Figure 7: Longitudinal cognitive assessments in 24 children with RE and controls. Follow-up after 2 years. Measurements of confrontation naming (A), immediate verbal memory (B) and digit symbol coding (D) all increase with time. A similar improvement is seen in motor function (C). Confrontation naming ability improves the most and immediate verbal memory the least. Despite these improvements the RE group does not reach equality in results with their normal peers. Extract from (Garcia-Ramos et al. 2015)

Larger studies with a prolonged follow-up reveal that some cognitive deficits remain in seizure remission A controlled study of 32 children with RE, over five years after seizure onset. They found that visuo-spatial memory improved to be comparable with controls but executive function (p=< .05), word comprehension (p=< .01) were significantly different at follow-up (Lindgren et al. 2004) where 71.8% were in seizure remission. One of the strengths of this study is that all of the children had stable and typical RE, yet interpretation of the results was difficult as 36% of the controls had evidence of poor performance in the auditory and verbal memory tasks. Furthermore, interpretation of the results is limited by the absence of follow-up EEG data to identify spike remission. The evidence is limited, but a longer follow-up would suggest that some cognitive functions improve and others remain in deficit, which may be a result of atypical RE.

There is some evidence to suggest that cognitive functions are better at improving in those with typical RE compared to atypical. An uncontrolled, longitudinal, prospective study divided 44 children with RE into a typical group (28 with a normal EEG background) and an atypical group (16 with slow-wave focus or asynchronous foci on EEG). The participants had twice-yearly neuro-psychology testing, with concomitant 2hr awake EEG with an additional overnight EEG every year (Metz-Lutz and Filippini, 2006). In seizure and spike remission, which was classified as being seizure-free with one year of normal waking and sleep EEG, all groups showed an improvement. However, the atypical group still had problems with verbal short-term memory (p=0.023), verbal learning (p=0.017) and reaction times in a continuous performance task (p=0.05). (Metz-Lutz and Filippini 2006). Despite the detailed methods of data collection, the raw results in this study are not presented in full, and this makes it difficult to obtain a good understanding of the results. Furthermore, the lack of controls hinders interpretation, and the presence of asynchronous RS foci, representing an atypical RE is a point for debate. Nevertheless, this study suggests issues with verbal-short term memory in seizure remission in atypical RE; other studies suggest that this deficit could be more prevalent.

There is evidence that children with typical RE may have problems with verbal memory and components of phonological awareness in seizure remission. In an uncontrolled longitudinal study of 23 children with RE, who were seen at least 12 months after baseline assessment were divided up at follow-up into those with active epilepsy (n=17) and those with seizure remission ≥ 12 months (n=6), with or without medication use, and a normal sleep EEG (Northcott *et al.*, 2006). In the whole group,

there were improvements in all cognitive scores at follow-up. When those with active epilepsy and seizure remission were compared, the group in remission performed significantly lower on the WRAML subtest called sentence memory. The study was uncontrolled, and the group with seizure remission was very small; however, there is some evidence that small deficits in either phonological processing or verbal STM are apparent in seizure remission. .Interestingly there are neurophysiological data to support these findings.

In a case-controlled, cross-sectional study of13 individuals with RE in seizure remission, aged between 15-23.8 years were assessed using neuropsychological testing and event-related potentials. The individual with RE had problems with receptive vocabulary (p=0.021), sentence assembly (p=0.001) and basic reading (p=0.014) (Monjauze et al. 2011a). Furthermore, in the event-related potential task, participants were asked to generate verbs in response to the auditory presentation of common nouns. In the RE group, this produced a large spatial shift in the evoked potential in the right frontal electrodes, which was not seen in controls. Interestingly the extent of this shift correlated with the Clinical Evaluation of Language Fundamentals (CELF) full expressive score (p=0.002) and basic reading (p=<0.001). The numbers studied were small, but there is some neurophysiological evidence that adolescents in seizure remission may struggle with verb generation and this may be due to aberrant cortical processing the following studies have shown that when compared to healthy controls deficits in phonological processing and reading may still be apparent in adolescents with Rolandic epilepsy in seizure remission. An analysis of other studies of individuals with RE in seizure remission appears to reveal this picture.

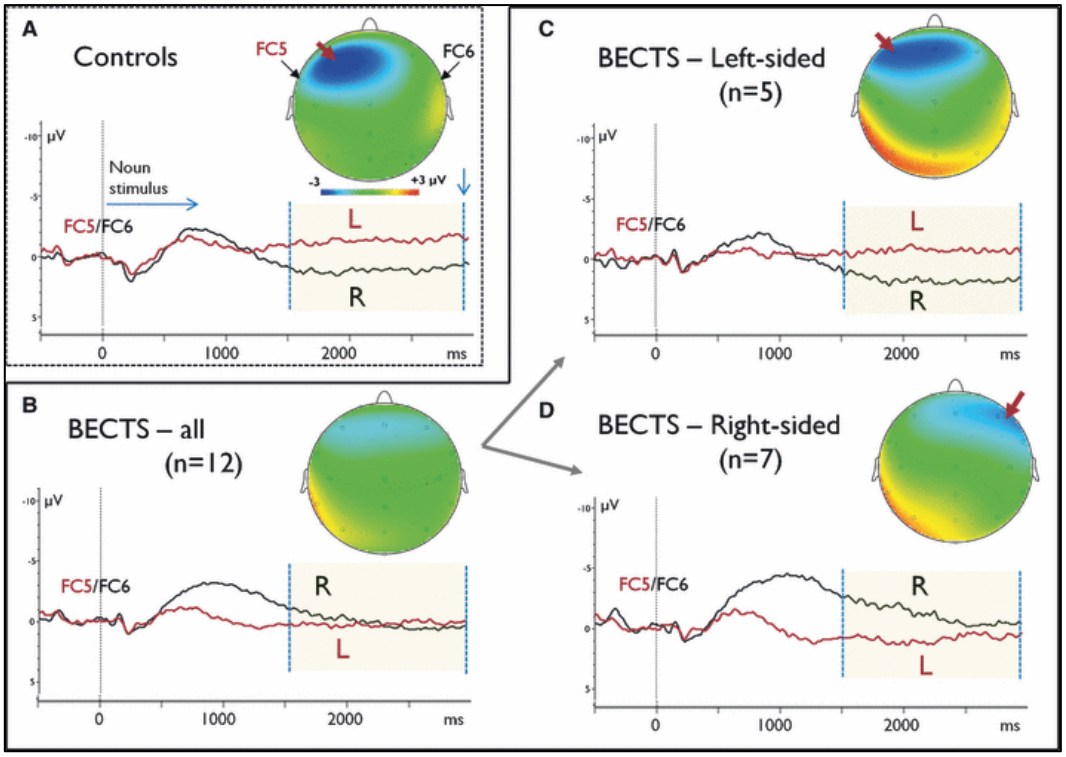


Figure 8: ERP group average waveforms recorded during the verb generation task from frontal electrode pairs FC5/FC6A) The control group shows a left-lateralized sustained negativity in the time window 1500–3000 ms after stimulus onset. Blue arrow pointing down at 3,000 ms indicates onset of tone cue instructing the participant to speak. Topographical map shows scalp distribution of mean amplitude in this interval (indicated by yellow shading) on the graph. B) Group average of total RE (BECTS) group (n = 12) shows loss of lateralized frontal negativity, C) whereas a subgroup of n = 5 RE participants shows typical left lateralisation (red arrow). D) Atypical right-sided lateralization was found in n = 7 RE individuals. Extract from Monjauze et al (2011a)

2.5.2 *Overview of cognition in seizure remission*

An analysis of eight studies where neuropsychology testing was used in children and adolescents in seizure remission reveals that there is an overall improvement in cognition (Table 9). The findings of this data were coded for the most prominent cognitive function used in the task (Table 10). The result of the coding provides evidence to support improvements in language, visual-spatial and verbal short- and long-term memory, executive functions and attention and motor abilities in seizure remission. However, the analysis identifies a distinct lack of data on speech, auditory processing and literacy. On the crucial question of whether children with RE cognitively improve to reach parity with healthy controls, the evidence is lacking. Nevertheless, there is some to support an improvement language, visuo-spatial STM, executive functions and attention and motor abilities. Finally, there is evidence to suggest persisting problems in seizure remission; this includes deficits in language, literacy, executive functions and attention and with a prominence of verbal short-term memory, and motor skills. It would appear that there is a general improvement in cognitive abilities in seizure remission; there is also the potential for cognitive deficits. Despite these deficits, they may do better than peers with other idiopathic epilepsies in seizure remission.

Study	N	Mean age	N in seizure remission (%)	N in spike remission	Controls	Improved cognition (longitudinal)	Cognition matches controls	Cognition worse than controls or norms
Metz-Lutz et al. (2006)	44	Around 12 years	100%	N/A	No	Corsi block (F) Rey's verbal learning (I)	Unclear	Reaction time on a continuous performance task. (J) Verbal short-term memory (H)
Northcott et al. (2005)	28	Unable to calculate	21.4%	6 had normal sleep EEGs	No	Sentence memory (G) Number letter (C) Verbal Memory Index (C) General memory index Receptive language (C) Concepts and following directions (C) Semantic relationships (C) Phoneme manipulation (C) Verbal motor production (A)	Unclear	Those in remission (n=6) performed worse at: Sentence memory (H)

Study	N	Mean age	N in seizure remission (%)	N in spike remission	Controls	Improved cognition (longitudinal)	Cognition matches controls	Cognition worse than controls or norms
Verrotti et al. (2011)	25	7-11 years	100%	100%	Yes	Peabody picture vocabulary test (C) Confrontation naming (C)	Performance IQ (E) Verbal IQ (C) Peabody picture vocabulary test (C) Confrontation naming (C)	None
Fillipini et al. (2013)	24	Unable to calculate	96%	18 were normal	No	Not reported	Unclear	One sd below: Five short term verbal memory deficits 2 with visual-spatial attention deficits. Two sd below: Five dyslexia (D) Three dysorthographia (K)
Garcia-Ramos et al. (2015)	24	10.5 at baseline tested	71.4%	N/A	Yes	Confrontation naming (C) Immediate verbal memory (H)	No	Confrontation naming (C) Immediate verbal memory (H)

Study	N	Mean age	N in seizure remission (%)	N in spike remission	Controls	Improved cognition (longitudinal)	Cognition matches controls	Cognition worse than controls or norms
		two years later				Grooved pegboard (K) WISC III coding (J).		Grooved pegboard (K) WISC III coding (J).
Hommet et al. (2001)	23	20	100%	100%	Yes	N/A	Verbal IQ (C)	Dual-task procedure: Right finger tapping with repetitive vocalisation (K)
Monjauze et al. (2011)	13	18.2	100%	None on awake EEG	Yes	N/A	Digit span (H) Phonologic awareness (C) Receptive grammar (C) Formulated sentences (C) Recalling sentences (H) Spelling (C)	Receptive vocabulary (C) Sentence assembly (C) Basic reading (D)
D'Alessandro et al. (1990)	11	15.4	100%	100%	Yes	Trail making test A and B (J) Stroop Colour Word Test (J) Barrage of letters (J)	Trail making test A and B (J) Stroop Colour Word Test (J) Barrage of letters (J)	None

Study	N	Mean age	N in seizure remission (%)	N in spike remission	Controls	Improved cognition (longitudinal)	Cognition matches controls	Cognition worse than controls or norms
						Semantic fluency (J) Bender Visuo-motor (K)	Semantic fluency (J) Digit span (H) Block tapping (F,) 15-word test (F) Bender Visuo-motor (K)	
Baglietto et al. (2007)	9	In those with sleep EEG spike remission 11 years old	100%	56%	Yes	Verbal IQ (C) Performance IQ (E) Bender Visuo-motor (K) Block tapping (F,) Trail making A +B (J), Boston naming test (C) Benton's Word Fluency (J)	Unclear	Cancellation tasks: Numbers (time and errors) (J) Stroop Colour word II (errors) (J) Spatial abilities: Street Gestalt test (E)

Table 9: Neuropsychological investigations of children and adolescents with RE in seizure remission. Studies are ordered with respect to their sample size. Included are mean age at follow-up, the percentage in seizure remission, the percentage with normal EEG, whether controls were used. Neuropsychology reported for improvements from baseline (longitudinal studies), no difference and significant difference with controls/norms. Each test has been coded for the main weight of cognition assessed. A: Speech, B: Auditory processing, C: Language, D: Literacy, E: Visuo-spatial, F: Visual-spatial memory short term memory (STM), G: Visual-spatial memory long term memory (LTM) H: Verbal STM, I: Verbal LTM, J: Executive function and attention K: Motor. Studies with composite findings have been removed, e.g. Lindgren et al. (2004). In Fillipini et al. (2013), only disabilities were coded.

Cognitive function	Improved cognition (longitudinal)	Cognition matches controls	Cognition worse than controls or norms
A. Speech			
B. Auditory processing			
C. Language	√√√√√	√√√√√	√√
D. Literacy			√√
E. Visuo-Spatial	√√	√	
F. Visuo-spatial short-term memory	√√	√	
G. Visuospatial long-term memory	√		
H. Verbal short-term memory	√	√√	√√√
I. Verbal long-term memory	√		
J. Executive function and attention	√√√	√	√√
K. Motor	√√√	√	√√√

Table 10: Coding of neuropsychological investigations in children and adolescents with RE in seizure remission. Data is extracted from table 2. The ticks represent studies which had supporting evidence. Blue shading indicates no available evidence. Orange shading: 3 or more supporting pieces of evidence. This overview demonstrates that language, executive function and attention and possibly motor abilities will improve in seizure remission.

Children with RE in seizure remission, tend to achieve better in adolescence and adulthood than those with other IECs in seizure remission. In a study of 42 adults who had RE as a child were contacted 29.5 ± 2.8 years after last clinical contact to investigate social outcomes. In this group, 78% graduated from high school, and 88% of those children went on to university, community college or technical college and 97% were in full-time employment (Camfield and Camfield 2014). The findings of this study may have been influenced by 50% of the cohort were on AED therapy (for an average of 3 years). Furthermore, quite a significant proportion, 24% could not be contacted on follow-up, and these may be the patients who have persisting cognitive and behavioural issues. These findings corroborate with the work of Loiseau *et al.*, Callenbach *et al.* and Blom and Heijbel who all found that children with RE had a favourable long-term prognosis and in some instances, these adults were outperforming their peers. (Blom and Heijbel 1982, Loiseau et al. 1983, Callenbach et al. 2009). These studies gathered cross-sectional data on social and academic outcomes, so it is unclear if neuro-psychological deficits remain. Also, it is unclear whether the studies have been asking the correct questions, for example, it would be good to know that these adults are performing to the same standards as other adults of a similar age and socio-economic background.

The evidence from the literature suggests that there is a three-layered remission in RE. Firstly there is the remission of seizures followed by inter-ictal spikes and finally a phase where there is a significant improvement in cognitive abilities. Seizure and spike remission appears to be sequential, whereas cognition can improve with or without seizure or spike remission. However, the rate of improvement in these children is still delayed, and they as a group perform worse than their age-matched peers up to 5 years after seizure onset. What is relatively clear is that these children with RE, as adults, can match their peer's inability, but this assumption is based on questionnaire data. There is a clear need for serial neuro-psychological data from adolescent and adults with RE in seizure remission at an individual and group level. This data will allow for the identification of how individual differences impact cognitive development and find at what point do these children cognitively match their peers, and whether do subtle deficits persist into adulthood.

2.6 Variation of cognitive problems

2.6.1 Familial cognitive problems

The previous sections have looked in detail at cognition in children with RE, but these cognitive deficits are not unique to them alone. There is a growing body of data to suggest that families of children with RE have similar cognitive problems all be it to a lesser extent. These deficits include auditory processing, language, speech and reading and working memory (Smith *et al.*, 2012; Verrotti *et al.*, 2013). Auditory processing problems appear to be prevalent.

Features of central auditory processing disorders (CAPD) appears to be prevalent in siblings and parents of children with RE. Smith *et al.*, investigated CAPD in a medium-sized controlled, cross-sectional study of 40 children with RE, 32 siblings and 71 parents. The control group consisted of 99 typically developing children and 31 adults. Using the SCAN test for auditory processing disorders they found that when compared to healthy controls, both children with RE (p=0.008) and siblings (p=0.039) and parents (p=0.005) were significantly different in the competing words subtest (Smith *et al.*, 2017). Furthermore, an atypical left ear advantage was seen in 25% of the children with RE, 30% of the siblings and to a lesser extent 3.4% of the parents (Smith *et al.*, 2017). This study neglects to report which children had spikes at the time of the SCAN testing. However, the findings would suggest that atypical processing is common in families with RE, and it persists into adulthood. Atypical auditory processing could be fomenter of other problems with speech development.

Speech sound disorder (SSD) is impairment in the development of speech motor control and this can be part of the developmental history of children with RE and their family members. A case-controlled, cross-sectional study of 55 children with RE, 67 siblings and 108 parents found that 37% of probands, 5% of siblings and 2% on parent controls had evidence of SSD (Clarke *et al.*, 2007). Evidence of SSD had an odds ratio with children with RE of 5.36 (95% CI: 2.40–11.96) (Clarke *et al.*, 2007). The association was not weakened when the SSD status of the child with RE was controlled for, suggesting that SSD is a prevalent cognitive deficit in RE. This study was weakened by its reliance on parents recalling features of SSD also there was a possibility for selection bias; however, it provides good evidence that SSD can be seen in families with RE. Moreover, it demonstrates that SSD might

have a stronger association with the epilepsy rather than part of a potential cognitive endophenotype. In the same study, dyslexia was investigated, and familial problems were prevalent.

In families of children with Rolandic epilepsy dyslexia or reading disability (RD) are present. In the Clarke *et al.* study*,* 55% of children with RE, 25% of siblings and 16% of parents had evidence of dyslexia. These findings suggest that there was an increased risk of having dyslexia in parents with a child with RE, odds ratio 2.84 (95%CI: 1.38–5.84) (Clarke *et al.*, 2007). This association was weakened when the RD status of the child with RE was controlled for, suggesting that the risk of dyslexia in families with RE is dependent on the status of the proband (Clarke *et al.*, 2007). Also, it raises the possibility that dyslexia in RE is likely to be due to genetic or environmental factors (Clarke *et al.*, 2007). This relationship would suggest that dyslexia could be a possible risk of developing RE, and it is not the result of having RE.

The following studies provide strong evidence that there is an overlap in the cognitive profiles of children with RE, their siblings and parents. In family members of children with RE, dyslexia, appears to be the dominant cognitive deficit, whereas SSD or features of CAPD are seen to a lesser extent. These deficits may suggest that there are some cognitive features which are specific to active epilepsy, rather than an overarching cognitive endophenotype.

2.6.2 **Cognitive overlaps**

This review has attempted to look each cognitive deficit in children with RE separately, whereas the reality a mixture of cognitive dysfunction which can overlap in particular reading problems. For example, Overvliet *et al.* found in 48 children with RE that there was a significant correlation between motor development and word (p=0.006) and sentence reading ability (p=0.03) (2011c). Although these findings were based on parents rating of motor-development, the data suggest that the severity of reading problems had an association with whether the child had delayed motor development. Vega *et al.* found in 108 children with RE found that reading disability was strongly associated with SSD (29%, OR 9.64, 95% CI: 2.45–37.21), ADHD (19%, OR 10.31, CI: 2.15–49.44) like the Overliet study the data was not directly measured but it would suggest that these traits strongly co-occur (Vega *et al.*, 2015). Another study investigated 22 children with RE and found difficulties in complex spatial perception and short and long term spatial memory, but this co-existed with arithmetic and orthography problems in a third of the children (Völkl-Kernstock, Willinger and Feucht, 2006). 31.8% of this cohort were treated with AEDs, which may have influenced the results. The amounts of studies which investigate the overlap of cognitive deficits in RE are small; however, they quite striking overlaps between different cognitive deficits suggesting multi-domain cognitive dysfunction. In particular, there is an overlap between reading and visual-spatial performance. As well as cognitive overlaps strengths can be apparent.

2.6.3 **A small proportion have a lack of deficits and cognitive strengths**

The lack of deficits and cognitive strengths are one of the most under-appreciated aspects of cognition research in children with RE. In an uncontrolled study of 33 children with RE, a task was used to assess the ability to write, read and perform arithmetic. In the RE group, overall performance was superior in two and average for six; this was despite one of the superior scorers having evidence of TCI. (Fonseca, Tedrus and Pacheco, 2007). It is important to note that the cohort was young mean age 6.9 years, and 21 of these children were non-medicated. Similarly, another study assessed 17

children with RE against controls matched for age, sex and school. Using a spatial learning task where children have to remember the locations of 9 cards, they found that 88% of the RE group achieved the highest score. Moreover, in story recall, 17% scored the highest score (Croona *et al.*, 1999). It could be the case that the spatial learning task has a ceiling effect, but story recall should be difficult for children with RE. Similarly, Neri *et al.* used the trail making test B, which assesses executive function in 53 children and teenagers with RE. They found that 10 had mid average, 1 had an upper average, and 1 had superior performance. This study had a mean group age of 10.9±1.7 years, which is old for a typical RE group (Neri *et al.*, 2012). Data on individual cognitive strengths are limited; however, the evidence would suggest that in a small proportion of children with RE strengths in academic skills, spatial memory and executive function can be apparent.

2.7 Conclusions

This review has attempted to provide a detailed overview of cognition in RE. Its aim was primarily for hypothesis generation for identifying potential cognitive issues in seizure remission. It has also tried to dissect the possible reasons why children with RE may have disordered cognition. In summary, the data would suggest that cognitive dysfunction pervades into every area of the cognition; however, there are areas of cognition which have a stronger evidence base. These include speech, language, phonological and auditory processing, reading, short- and long-term verbal memory, attention and executive function and motor problems. These cognitive deficits improve with time, but it is unclear whether they reach parity with healthy controls. Part of this may be due to familial influences such as speech sound disorder, central auditory processing disorder and dyslexia which have a high prevalence in these families of children with RE; these are very likely to contribute towards the RE cognitive phenotype. The data on diurnal spikes and seizure phenomena influencing cognitive deficits other than attention and motor and sensory phenomena are weak, and it is quite likely that their role is overstated. Conversely, the density of nocturnal spikes may be associated with semantic language and reading, but a direction of interaction is not well defined. The findings of this review suggest that there is an underlying cognitive dysfunction within the brain, similar to what is seen in family members, but this dysfunction is on a greater scale. This idea will be explored in more detail within this section, but first, the findings of the review will be explored.

2.7.1 The use of intelligence quotient metrics in idiopathic epilepsy is unlikely to identify cognitive deficits

The reliance on intelligence quotient (IQ) to assess children with idiopathic epilepsies is not effective, and this is especially the case in RE. In children with RE moderate to severe cognitive deficits can be apparent despite a normal IQ. Possible reasons for this are one, children with RE can have above average to superior intelligence and two, IQ subtests are non-specific for cognitive problems, and the tests are bad at identifying children with a specific learning deficit (Restori *et al.*, 2009). Ideally, IQ testing should not be used to assess children with RE, however, if it needs to be used the best practice is to compare with a control group matched for age, sex and socioeconomic background (i.e. school friends). This review would also recommend that individual analysis rather than group analysis is more desirable. This is because there is a large variation in cognitive ability in children with RE, which includes normal and above-average intelligence.

2.7.2 A mixed cognitive picture

One of the problems within the RE literature is the attempt for some of the researchers to seek evidence of a benign disorder and for others to try and find cognitive issues to be used as biomarkers. A biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.” (‘Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework’, 2001). The literature would suggest that there is no cognitive biomarker for RE but nor is it a benign disorder. This review would suggest that cognition in children with RE is highly variable spanning from below average to superior abilities. Deficits can be multi-domain involving speech, language, phonological and auditory processing, reading, verbal short- and long-term memory, attention and executive function and motor and sensory processes. Normal and superior abilities can also be seen in some, in reading, writing, arithmetic, spatial memory and executive functions, some of the same areas where deficits are seen in others.

The evidence of cognitively normal children in RE warrants further investigation. It is possible that they are the result of poor recruitment such as patients that have a RE diagnosis after one seizure or a child who is misdiagnosed from EEG findings. These methodological problems may be a small part of the problem, but most studies reviewed used the strict ILAE criteria for diagnosis. Subtle deficits also appear to be unlikely as many cohorts of children with RE have been rigorously tested. Variations in access to education may be possible, but there is no evidence to suggest that this is the case. There are two possibilities for this phenomenon, one; it is possible that it could be due to beneficial plastic changes as a result of the seizure disorder or two, they have precocious brain development which is resulting in a seizure disorder. Investigating this sub-group of children may help find neuronal targets for therapies to aid those children with RE and cognitive dysfunction. It would also appear that the risk of cognitive problems is derived from the genetic background of their families.

2.7.3 **Familial cognition matter**

Understanding the cognition of families with children with Rolandic epilepsy is very important as this can help dissect the probable causes for certain cognitive problems. In essence, what is family-specific, and what is epilepsy specific? This review has demonstrated that speech sound disorder, central auditory processing disorder and dyslexia have a high prevalence. These deficits can be classified as background cognitive deficits, which would be in keeping with the idea of endophenotype in families of children with Rolandic epilepsy. This background cognitive deficit can be apparent without spikes on scalp EEG or seizure phenomenon (Clarke et al. 2007, Smith et al. 2012b). Other than a small study by Verotti of children with RE and their siblings (Verrotti *et al.*, 2013). There are no detailed family studies which have investigated motor and coordination, visuo-spatial abilities, attentional and memory problems within family members. The lack of interest in these cognitive domains within family members could suggest that these deficits are epilepsy specific and are related to spikes and seizures. If this is the case, there is an expectation that epilepsy specific deficits to improve with spike and seizure remission but the family specifics one to remain. The idea of splitting the cognitive functions needs to be approached with caution because as all of the family studies were

group analyses, it is unknown how similar the deficits are between and the probands both in the range of problems and the severity of them. The absence of this data is a gap in the literature knowledge that a paired proband-sibling analysis could remedy (Smith et al. 2012b). Nevertheless, the use of the proposed model can help explore the cognitive effect of spikes with more detail.

2.7.4 **Spikes appear to be associated with specific cognitive problems**

This review would suggest that there is an association between the appearance of spikes on the EEG and motor, sensory and attentional issues. Large nocturnal RS densities and fixed RS appear to be associated with poorer semantic language, reading and writing disorders and attentional deficits. Studies in children with RS but no seizures would support these findings. Holtmann *et al.* found in a group of children of 48 children with ADHD than those with spikes (33.3%) yielded poorer results on most continuous performance task measures suggesting poorer attentional abilities (Holtmann *et al.*, 2006). Furthermore, Carlsson *et al.* found in 15 children with dyslexia and incidental CTS were more likely to make reading mistakes and had a propensity for impaired attention compared to those without CTS (Carlsson *et al.*, 2000). Overall, it would appear that spikes make poor cognition worse, and this might be due to the effect that they have on the cortex.

In the children with RE and diurnal spikes, it indicates that the cortex is already in a hyper-excitable state. This state potentially can disrupt cognitive processes within the cortex. The altered cortical function has been demonstrated in fMRI studies by recording changes in blood oxygen level-dependent (BOLD) signal at the time of RS. A study found that the RS discharge had positive correlations with the bilateral inferior frontal gyrus and left inferior parietal lobe, supramarginal gyrus and caudate (Xiao *et al.*, 2016). These findings demonstrate the extent of the discharge outside of the central sulcus, and it is possible this may disrupt cognition. There is evidence of an altered BOLD signal in association with RS; however, this review has shown that diurnal spikes do not directly lead to an impairment of cognition in a time-locked fashion. Moreover, this review has demonstrated that spike remission is not required for cognition to improve, suggesting that spikes are unlikely to hinder

neurodevelopment. These findings would suggest that the spike in itself is the wrong measure, and it is the underlying cortical excitability, which is causing cognitive dysfunction.

There is a possibility that the RS in children with RE is an irrelevant feature, and it is the underlying hyper-excitable cortex, which is the main problem. Furthermore, the RS cannot be equated with true spikes (<70ms) seen in other epilepsies. Positron emission tomography (PET) has demonstrated that in children with symptomatic epilepsies, fluoro-deoxy-glucose (FDG) PET imaging will produce an area of hypo-metabolism over the epileptogenic region. However, this phenomenon is absent in children with RE (Van Bogaert et al. 1998). Similarly, EEG-functional MRI studies suggest that centro-temporal spikes do not elicit a canonical BOLD response. Masterton *et al.* demonstrated that in children with RE, there was an increase in BOLD before spike generation and then a pronounced undershoot of the signal (Masterton *et al.*, 2010). They suggested that this response may reflect a cortical stimulus initiating the spike followed by reduced neuronal activity. Studies of symptomatic epilepsies demonstrate that true spikes follow the canonical model, and any non-canonical responses are a result of artefacts or propagated epileptiform activity (Lemieux *et al.*, 2007). The lack of evidence for a metabolic focus and the altered BOLD response would suggest that spikes in RE are quite different from those with symptomatic epilepsies, which may explain the complicated cognitive picture.

2.7.5 There is a weak relationship between seizures and disordered cognition in the idiopathic epilepsies of childhood

The evidence is weak for seizures impacting cognition, Northcott's large study did not find any link between cognition and seizure frequency which would suggest that the seizures are most likely benign (Northcott *et al.*, 2005). There is evidence that atypical seizure types may result in adverse cognitive outcomes; however, these studies are cross-sectional, so it is unclear what is cause and effect. As the link is unclear, it could be that cognitive issues are preceding epilepsy and those that have a greater level of cognitive dysfunction are more susceptible to generating seizures.

2.7.6 Altered cognition is evidence of aberrant neurodevelopment

Cognitive problems in children with RE seem most likely to be apparent in the background as a result of abnormal neurodevelopment before the development of the epilepsy. As demonstrated in this review, cognitive problems can be apparent prior to the first seizure, which would suggest that brain structure and function are already abnormal. Possible reasons for impaired cognition include, inter-ictal spikes influencing cognition before the first seizure, subtle unnoticed seizures or aberrant neurodevelopment, which lead to cognitive problems. This review should dispel the idea that seizures impact cognition in RE, it would also suggest that the underlying hyper-excitable cortex rather than the spikes are the main factor influencing cognition. There is a distinct lack of evidence to suggest a relationship between spikes and cognitive abilities over time. Indeed cognition, on the whole, is seen in improve. Therefore, a new hypothesis is that the main cause of cognitive problems is aberrant neurological development which alters the brains structure. There are a few factors identified in this review which support this idea.

Familial cognition, delayed development and abnormal functional neuroimaging are all potential indicators of a neuro-developmental problem. The similarities between sibling and parents of children with RE would highlight the likelihood of genetic cause. It is quite likely that the genetic effect is occurring in early development; this is because speech and motor delay can be seen in children with RE. This is quite likely to be reflecting abnormal neurodevelopment as this phenomena can be seen in other disorders such as ADHD (Gurevitz *et al.*, 2014), dyslexia (Viholainen *et al.*, 2006), autism (Ming *et al* 2007) specific language impairment (Shriberg, *et al* , 1999) and developmental coordination disorder (Zwicker *et al.*, 2012). Finally, this review has demonstrated that brain function, as measured by neurophysiology, is altered in children with RE. Despite this evidence, most of these studies are cross-sectional, and it is unclear whether they are plastic changes, or they are apparent at seizure onset. As mentioned, there are links between RE and other neurodevelopmental disorders, in particular, specific learning disabilities.

2.7.7 Be aware of developmental coordination disorder and other specific learning disabilities

The range of cognitive problems are vast, and there is strong evidence to support dysfunction in speech, language, phonological and auditory processing, reading, verbal short- and long-term memory, attention and executive function and motor and sensory systems. There is also some evidence for dysfunction in visual-spatial systems. Regardless of the cognitive domain, these deficits can impair the child's ability to learn, and so these deficits are in keeping with the idea of specific learning disorders (SPLD). The SPLDs include developmental coordination disorder (DCD), dyslexia, developmental language disorder (DLD), central auditory processing disorders (CAPD) and attention deficit hyperactivity disorder (ADHD). This review would suggest that a child with RE could have none or one or more of these disorders. However, if a child with RE has an SPLD, it is most likely to be developmental coordination disorder or dyslexia. Despite the high likelihood of having an SPLD, it appears that the features of these disorders remit in seizure remission.

2.7.8 Features of dyslexia remain in seizure remission

Despite improvements in cognition focal deficits may still be apparent. These focal deficits appear to be predominantly in the reading, verbal memory and phonological abilities. These features are common to dyslexia (Peterson and Pennington, 2015). These findings would suggest that dyslexia's may be apparent in seizure remission, and there is a need for educational support. It is unclear if these deficits persist into adulthood; however, features of dyslexia in parents of children with RE would suggest that this is likely.

2.7.9 **Potential cortical substrates for cognition**

The range of cognitive problems with no “hard” neurological signs would rule out the primary motor and sensory cortices. Similar to the other idiopathic epilepsies of childhood, it would suggest that association cortices are the most likely sources of the dysfunction. The dichotomy of family and epilepsy specific problems can be used to explore potential cortical regions.

Family specific cognitive issues in children with RE include dyslexia, speech sound disorders and auditory processing disorders. There are cortical regions which could potentially be involved in these disorders. In dyslexia, the left angular gyrus (AG) is a likely candidate as damage to this region can lead to the loss of capacity to read and write words (Geschwind, 1965). There is evidence to suggest that the AG is involved in reading when semantic associations are made (Price and Mechelli, 2005), this brain region that helps with the comprehension of text, one of the main problems for people with dyslexia (Tunmer and Greaney, 2010). Closely linked to reading, are speech and phonology; however, the potential cortical substrate for this is located with the frontal lobe.

Problems with speech and phonological processing in RE could be due to the disorder within the left inferior frontal gyrus (IFG). This region is known as Broca's area; this is a motor association area and has been historically linked with expressive language problems and speech dyspraxias (Pearce, 2009). This brain region appears to be associated with individuals with speech apraxia as a result of stroke (Hillis *et al.*, 2004) Functional neuroimaging has implicated the left IFG in word production, semantics and phonological processing (Poldrack *et al.*, 1999). It also has a role to play in verbal fluency, which is a measure of executive function (Costafreda *et al.*, 2006). Closely linked to speech is auditory processing.

The most likely place for auditory processing problems in children with RE is the auditory association areas within the superior temporal gyrus (STG). This brain region contains Wernicke's regions; when a lesion is found in this region, the patient will have receptive aphasia (Hickok and Poeppel, 2007). This region is also said to be involved in the processing of auditory timing and the deciphering of speech in background noise which can be aberrant in individuals with central auditory processing disorders (Bueti, van Dongen and Walsh, 2008; Vander Ghinst *et al.*, 2016). Finally, damage to the

left STG can lead to the reversal of the right-ear advantage in dichotic listening, the same phenomenon which is seen in some children with RE (Maffei *et al.*, 2017).

An interesting feature of these brain regions is that there are links between them via white matter association tracts. Between the angular gyrus and the inferior frontal gyrus is the third branch of the superior longitudinal fasciculus and between the angular gyrus and superior temporal gyrus is the inferior longitudinal fasciculus (Seghier, 2013). The arcuate fasciculus is another large white matter tract, and this connects the inferior frontal gyrus, the parietal lobe and the superior temporal gyrus (Rilling *et al.*, 2008). Overall, there is a connected language network across the left hemisphere, which is a prime candidate for familial background cognitive problems in children with RE.

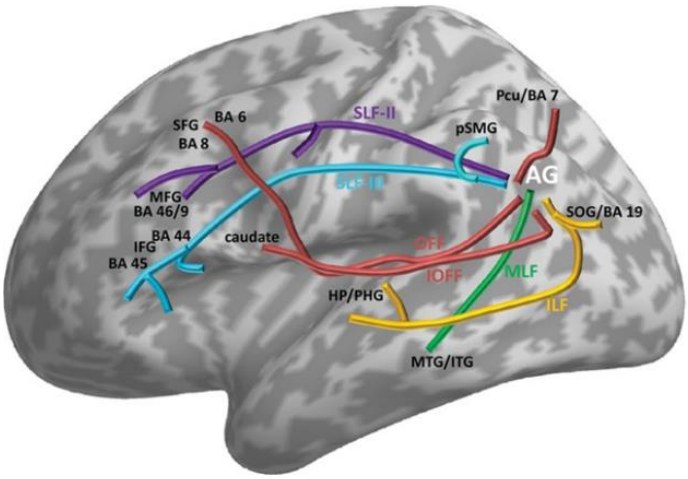


Figure 9: Schematic illustration of association white matter tracts which connect the angular gyrus to cortical regions.Tracts include (red) occipito-frontal fasciculus (OFF) and inferior occipito-frontal fasiculus (IOFF), (purple) superior longitudinal fasciculus II, (blue) superior longitudinal fasciculus III, (green) middle longitudinal fasciculus (MLF) and (yellow) inferior longitudinal fasciculus (ILF). Seghier et al (2013)

Epilepsy specific cognitive issues include attention, verbal memory, visuo-spatial skill and motor dysfunction. These cognitive issues are difficult to isolate to specific neurological substrates; however, there is one region which has the ability to integrate all of these cognitive variables. The inferior parietal lobe which is a multi-modal association area.

The inferior parietal lobe processes multiple cognitive domains, the same domains which are in deficit in children with RE. In attention research, transcranial magnetic studies have revealed that the right

inferior parietal cortex is implicated in visual target detection and reorienting (Chambers *et al.*, 2004). In motor function, animal data suggest that the inferior parietal lobe contain several areas involved in the control of arm, hand, face and eye movements (Fogassi and Luppino, 2005). In verbal memory, inferior parietal lesions have been implicated in phonological storage deficit (Baldo and Cronkers, 2006). Human functional neuroimaging studies provide evidence that semantic language problems can be visualised in the inferior parietal lobe (Binder *et al.*, 2009). Finally, the inferior parietal lobe is involved in the integration of visual information from both the dorsal and ventral streams (Singh-Curry and Husain, 2009). These findings are in keeping with the range of cognitive functions, many of which are impaired in children with RE would suggest that this region may be one of the hubs for the epilepsy.

2.7.10 **A potential hub for ictogenicity**

The cognitive data would suggest that a potential epileptogenic hub within in children with RE is within the inferior parietal lobe where. This section will present the evidence for this proposal; the first evidence is from evolutionary biology.

Rolandic epilepsy is unique to humans, which would suggest that an ictogenic hub is within a brain region which is unique to humans. The inferior parietal lobe is larger in humans compared to apes and monkeys, which is because of the angular gyrus, which has no homologue in non-humans (Culham and Kanwisher, 2001). Furthermore, the angular gyrus is the hub of several long association tracts; some of these tracts are none apparent or smaller in apes. Altered white matter tracts include the SLF III which is right lateralised in humans (Hecht *et al.*, 2015) and the arcuate fasciculus which enlarges up the evolutionary tree from macaques to humans (Thiebaut de Schotten, Dell'Acqua and Valabregue, 2012). Finally, compared to other brain regions, the inferior parietal lobe has less radial fibre bundles, and as a result, there is a distinct reduction in myelination (Glasser and Van Essen, 2011). Evolved brain structure may have predisposed some humans to develop idiopathic focal epilepsies, and this may be due to the nature of myelination of the inferior parietal lobe.

Neuroimaging data in children with RE would suggest that some of the abnormal myelination is occurring within the inferior parietal lobe and its connections. Potentially, abnormal white matter development includes regions of higher mean diffusivity (MD) in the left inferior parietal lobe (Ciumas *et al.*, 2014) and low fractional anisotropy (FA) and high MD in the left superior longitudinal fasciculus part of which, the SLF III, connects with the inferior parietal lobe. (Kim *et al.*, 2014; Xiao *et al.*, 2014). Furthermore, structural neuroimaging found regions of increased grey matter volume in the left parietal lobe in individuals with RE (Pardoe *et al.*, 2013; C. Luo *et al.*, 2015). The findings of these studies may be evidence of impaired myelination (Natu *et al.*, 2018). Interestingly, some symptomatic epilepsies with seizures similar to RE seizure semiology, indicate the parietal lobe.

There are several studies of individuals suffering from symptomatic seizures with a RE semiology where the lesions have been seen within the parietal lobe. Salanova *et al.* investigated 82 patients with non-tumour related parietal lobe epilepsy. They found that 52 (63.4%) had a semiology which

includes tingling and numbness, a common feature of the RE seizures (Salanova *et al.*, 1995). Furthermore, in a study of 24 children who had surgery for intractable symptomatic RE, 20.8% had lesions which were either within or extended into the parietal lobe (Behdad *et al.*, 2009). Moreover, there are case studies of symptomatic epilepsies with pure salivatory seizures and these have been associated with a thicker right parietal cortex (Nascimento *et al.*, 2016). There are several pieces of evidence to suggest that the inferior parietal lobe may produce semiologies of RE seizures.

Overall, there are cognitive, evolutionary, imaging and lesional data, which would suggest the parietal lobe, in particular, the inferior parietal and angular gyrus may have some involvement in the ictogenic network within children with RE.

3 A systematic review of neuroimaging studies in Rolandic epilepsy

3.1 Introduction

Rolandic epilepsy (RE) is a prevalent (15% of all new cases) childhood epilepsy (Camfield and Camfield, 2002; Watanabe, 2004; Mellish *et al.*, 2015) which is often associated with neurocognitive impairment. Cognitive problems can present prior to, or following their diagnosis of epilepsy and appear to have no relation to the frequency of seizures (G. M. Overvliet *et al.*, 2011; Vega *et al.*, 2015). These problems predominantly involve speech (Pal *et al.*, 2010), language (Overvliet *et al.*, 2013), auditory processing (Smith *et al.*, 2017) and reading (Smith *et al*., 2015). Other neurocognitive problems include attention (Kavros *et al.*, 2008), memory (Verrotti *et al.*, 2014), motor function (Ayaz *et al.*, 2013) and visuospatial skills (Völkl-Kernstock *et al*., 2006). Furthermore, it is unclear whether these cognitive deficits persist after seizure remission (Thierry Deonna *et al.*, 2000; Northcott *et al.*, 2006; Monjauze *et al.*, 2011). This is because there is evidence that similar neurocognitive problems can be apparent in both seizure-free sibling and parents of children with RE (Bali *et al.*, 2007; Clarke *et al.*, 2007; Smith *et al.*, 2012; Verrotti *et al.*, 2013). Although the reported neurocognitive problems in RE are diverse, group neuroimaging analyses may be able to reveal subtle abnormalities in brain structure that could explain such problems.

Routine magnetic resonance imaging (MRI) rarely detects any pathogenic/pathological abnormalities, and incidental findings are comparable to those in normal children (Boxerman *et al.*, 2007). Furthermore, several neuroimaging studies have tried to gain insight into brain abnormalities, but these have been sub-optimal and have produced findings of variable quality, for example, using CT scans (Gelisse *et al.*, 1999), low Tesla MRI (Kanemura and Aihara, 2009), large slice thicknesses (Lundberg *et al.*, 1999), lacking controls (Eeg-Olofsson *et al*., 2000) or retrospective clinical neuroimaging which may be affected by the aforementioned problems (Bajic *et al.*, 2009).

High-quality research MRI has produced interesting findings. In particular, a key longitudinal controlled study demonstrates delayed neurodevelopment: Garcia-Ramos and her colleagues used Freesurfer (Fischl, 2012) neuroimaging software to reveal significant differences in cortical thickness and subcortical volumes. In addition, they produced evidence to suggest that normal temporal changes in cortical thinning in children with RE occurred more slowly than among healthy controls (Garcia-Ramos *et al.*, 2015). Associated cognitive data indicated that the development of cognitive

abilities over time was also delayed. However, out of the 65 participants with cognitive data, only 43% of these were scanned.

This review aimed to identify all of the quantitative controlled MRI studies in children with Rolandic epilepsy and from this collection identify regions in cortical and subcortical grey and white matter which may have a role to play in cognitive dysfunction. Moreover, there is a need to understand how grey and white matter variables change over time. This study searched for cross-sectional and longitudinal controlled studies with quantitative findings in order to identify the highest-quality evidence. The search included both T1 structural analyses of cortical and subcortical grey matter and diffusion-weighted image (DWI) studies of white matter myelinated regions and tracts. Synthesis of these data should allow the assessment of whether qualitative differences found in the earlier literature are consistently observed. These include, asymmetrical hippocampi (Lundberg *et al.*, 1999; Bajic *et al.*, 2009), lateral ventricular dilation (Gelisse *et al.*, 2003), reduced frontal lobe volume (Kanemura and Aihara, 2009) and small regions of defective myelination in the frontal and temporal lobes (Lundberg *et al.*, 1999).

3.2 Methods

The methodology used in this review is based on the work of Eggers, Smith and Altman (2008) and Boland, Cherry and Dickson (2013). The strategy of this review was to 1) attempt to identify all relevant research, 2) make judgements about the quality of the literature, 3) systematically synthesise the findings of studies of acceptable quality and 4) makes judgements about the research questions (National Health and Medical Council, 2000).

3.2.1 Search Strategy

A multi-layered search strategy was utilised. First, the Prospero and Cochrane databases of systematic reviews were searched using the Medical Subject Headings (MeSH) search term "Rolandic epilepsy" on 18/04/2017. Secondly, the MeSH terms "Rolandic epilepsy" and "magnetic resonance imaging" were used to search three major databases (Pubmed, Ovid, and Scopus) on 21/04/2017. Thirdly, a focussed search on the Web of Science database used the MESH term "Rolandic epilepsy" and the search terms "TOPIC: (rolandic epilepsy) AND TOPIC: (diffusion tensor imaging) AND TOPIC: (DTI) AND TOPIC: (control)" and "TOPIC (rolandic epilepsy) AND TOPIC: (cortical) AND TOPIC: (control)". TOPIC was used as it searches titles, abstracts, authors keywords and *KeyWords Plus*® a Web of Science feature which augments traditional keyword retrieval. Even though the majority of the neuroimaging literature has been produced over the last decade, there were no date restrictions to the searches. The bibliographies of all of the identified papers were also searched in order to find additional papers. Finally, a search for grey (unpublished) literature involved investigating archives at King's College London and requesting all of the major RE research groups to supply grey literature for the study.

3.2.2 Inclusion criteria

Papers and abstracts were included if they fulfilled the following criteria: 1) they described T1 structural studies or 2) diffusion-weighted imaging studies 3) the data they describe is quantitative 4) 5) they were group studies and 6) they were controlled. The participants had to have a diagnosis of rolandic epilepsy which was either focal or secondary generalised and had to fulfil the criteria set by the International League Against Epilepsy ('Proposal for Revised Classification of Epilepsies and Epileptic Syndromes.', 1989). No studies were excluded based on diagnostic attributes such as seizure frequency, anti-epileptic drug therapy, inter-ictal EEG spike density or location, duration of epilepsy, age of first seizure or cognitive deficits.

3.2.3 Grouping of studies

The cross-sectional and longitudinal studies were grouped into the following categories:

1) Grey matter: Structural analysis of the cortex and subcortical structures from T1 weighted magnetic resonance images.

2) White matter: Structural analysis of myelinated brain regions and tracts using diffusion-weighted magnetic resonance images.

3.2.4 **Quality check**

Selection

1) Is the case definition adequate?
   a) Yes, with independent validation *
   b) Yes, e.g. with record linkage or based on self-reports
   c) No description

2) Representatives of the cases
   a) Consecutive or obviously representative series of cases *
   b) Potential for selection biases or not stated

3) Selection of Controls
   a) Community controls *
   b) Hospital controls
   c) No description

4) Definition of controls
   a) No history of disease (endpoint) *
   b) No description of source

Comparability

1) Comparability of cases and controls on the basis of the design and or analysis
   a) Study controls for age *
   b) Study controls for gender or handedness *

Figure 10: The modified Newcastle-Ottawa score used for quality assessment. Only selection and comparability sections were used. Furthermore, the covariates of interest were age, gender and handedness. The maximum score in this assessment was six points. Criteria with a (*) had to be fulfilled to obtain a star. If there was any uncertainty, the point was not awarded.

The results of the searches after the implementation of the inclusion criteria were assessed for quality. Quality assessment was performed using a modified Newcastle-Ottawa Quality Assessment Scale for Case-Control studies (2014). The selection and comparability sections were used with a maximum score of six stars (see Figure 10). The exposure sections of the Newcastle-Ottawa scale were irrelevant to this study. Studies with less than three stars were excluded from the synthesis of results.

3.2.5 **Extraction of data**

Data were extracted from the resulting papers and abstracts. The details from T1 MRI studies of brain regions or structures where the study's authors detected a significant difference in cortical thickness (mm), or volume (mm^3). This includes subcortical structures and statistically significant differences in shape. In the diffusion-weighted MRI studies, data were extracted for brain regions or tracts where the reporting authors detected either a significant difference in fractional anisotropy (a scalar value between 0 and 1) or mean diffusivity (mean of three eigenvalues which form the tensor). Studies which included multiple groups were divided into their constituent groups and their data extracted.

3.3 Results

Two hundred and twenty-two papers were identified in the search process. After exclusion criteria were applied a total of 13 papers remained. Eight papers were related to the structural analysis of grey matter, and five were white matter studies. A PRISMA flow diagram

Figure 11) shows the search results and subsequent screening.

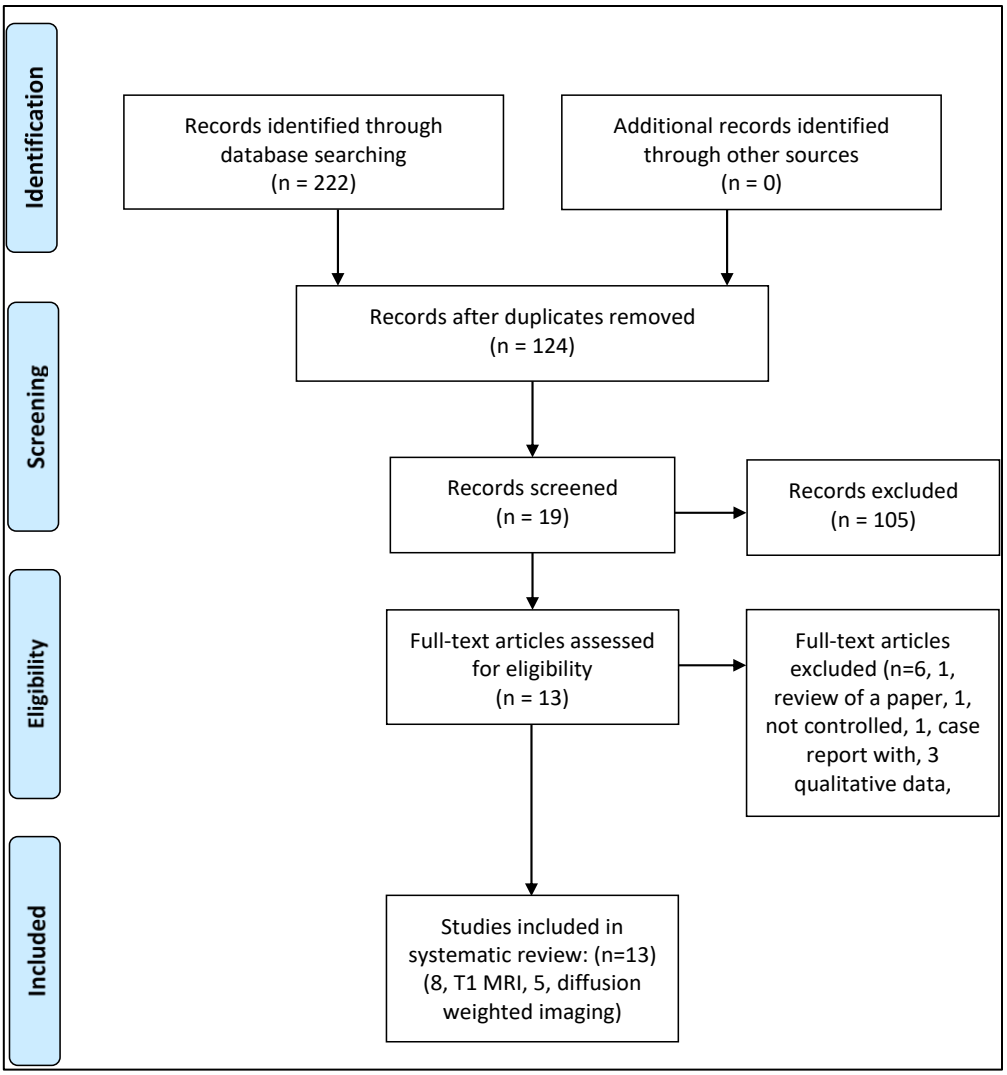


Figure 11: A PRISMA flow chart of the systematic review search process (Liberati *et al.*, 2009)*. Included in the study were 8 T1 structural neuro-imaging studies and 5 diffusion-weighted image studies.*

The following results sections will be divided into grey matter (cortical volume and thickness measurement) and white matter (fractional anisotropy and mean diffusivity measurements).

3.3.1 Grey matter studies

3.3.2 Quality check

Study	Is the case definition adequate?	Representatives of the cases	Selection of controls	Definition of controls	Comparability of cases and controls on the basis of design and analysis	Stars (out of 6)
Kohrogi & Mitsudome (1993)	Not reported	Not reported	Unclear	1 Star: No history of disease	Unclear	1
Kanemura et al (2011)	Not reported.	Unclear, some children had cognitive regression.	Not reported	Hospital controls	No covariates	0
Lin et al (2012)	1 Star: Independent validation. Based on clinical and EEG data	Unclear. Nocturnal generalised tonic-clonic seizures only. Simple partial during waking hours only.	1 Star: First degree cousins	1 Star: No history of disease.	2 Stars: Age and sex as covariates.	5
Overvliet et al (2013)	1 Star: Independent validation. Based on clinical and EEG data	1 Star: Representative series of cases	Unclear	1 Star: No history of disease.	1 Star: Sex as a covariate	4
Pardoe at al (2013)	1 Star: Independent validation. Based on clinical and EEG data	1 Star: Representative series of cases	Only reported clearly in group A	Only reported that group A were healthy.	2 Stars: Age, sex, brain volume and group as covariates.	4 (6 for Group A)
Garcia-Ramos at al (2015).	1 Star: Independent valiadation. ILAE Classification	1 Star: Required a diagnosis of epilepsy within the last 12 months	1 Star: Healthy first-degree cousin controls matched for age and gender	1 Star: No history of disease	2 Stars: Multivariate analysis of covariance (MANCOVA) with age, gender and intra-cranial volume (ICV) as covariates.	6
Kim et al (2015)	1 Star: Independent valiadation. ILAE Classification	1 Star: Representative sample from an epilepsy clinic	Unclear	1 Star: No history of disease.	1 Star: Handedness as covariate	4
Luo et al (2015)	1 Star: Independent validation. Based on clinical and EEG data	Excluded patients with attention deficit hyperactviity disorder (ADHD)	Unclear	1 Star: No history of disease.	2 Stars: Age, sex and whole brain volume as covariates	4

Table 11: Grey matter study quality check results. Sections: Yellow criteria fulfilled (1 Star); white criteria unfulfilled. Articles >3 stars= blue, <3 stars = red. Studies with less than 3 stars were excluded from the analysis. There was only one study with the full 6 stars.

Table 11 contains the results of the quality check. The range of quality was wide (0-6 stars). The mean was 3±2 stars. Only one study, Garcia-Ramos completely fulfilled all of the criteria (2015). Group A of the Pardoe study also fulfilled all of the criteria (Pardoe *et al.*, 2013). Quality was lost in creating a representative sample of children with RE and the reporting of how controls were selected.

Two papers (Kohrogi and Mitsudome, 1993; Kanemura *et al.*, 2011) failed the quality check and therefore, were not included in any further analysis.

3.3.3 **Participant characteristics**

Studies	Star Rating	Number of RE	Number of controls	RE Mean Age (years±SD)	Control Mean Age (Years±SD)	RE Male	Control Male	RE Female	Control Female	RE and Control Male Ratio	RE Handedness (Right/Left/Ambidextrous/Unknown)	Control Handedness (Right/Left/Ambidextrous/Unknown)
Lin et al (2012)	5	13	54	10.2±1.4	13.2±3	8	24	5	30	3.00	Not reported	Not reported
Overvliet et al (2013)	4	24	24	11.3±1.9	10.6±1.8	15	14	9	10	0.93	20/3/1/0	22/2/0
Pardoe et al (2013) (Group A)	6	16	20	9.3±1.6	9.7±1.6	11	12	5	8	1.09	16/0/0/0	18/2
Pardoe et al (Group B)	4	9	35	15.8±2.3	16.9±4.6	3	17	6	18	5.67	8/1/0/0	Not reported
Pardoe et al (Group C)	4	10	20	22.7±2.7	23.7±3.4	3	8	7	12	2.67	9/1/0/0	Not reported
Garcia-Ramos et al (2015).	6	13	24	10.3±1.9	11.3±2	7	11	6	13	1.57	Not reported	Not reported
Kim et al (2015)	4	20	20	7.5±1.5	7.4±1.5	14	14	6	6	1.00	14/2/0/4	14/2/2/2
Luo et al (2015)	4	21	20	9.12±1.51	9.26±1.96	13	10	8	10	0.77	21/0/0/0	20/0/0/0

Table 12: Participant characteristics in grey matter neuroimaging studies. Included are sample sizes, mean age, sex and handedness. Male sex ratios between RE and controls are included. The first two columns (coloured in light blue) contain the studies name and quality rating.

Table 12 contains the number of participants and their characteristics. One hundred and twenty-six participants with RE (mean number 16±6.75) were scanned across the six studies. For ease, the following summary of the studies includes the Pardoe *et al*. study as three separate studies due to their use of three different groups. The range of mean ages across the studies was large between 7.5 to 22.7 years. More males with RE (total 74, mean 9.25±4.74) were recruited compared to the females (total 52, mean 6.5±1.41). The sex differences resulted in a ratio of 0.70 females to every male. In comparison, there were two hundred and seventeen controls (mean 27.13±11.99) scanned across the studies. The mean age of the controls was similar to the participants with RE and ranged from 7.4 to 23.7 years. The sex ratio was also close to an even distribution of 110 males to 107 females (0.97).

Handedness was under-reported across all of the studies for both participants and controls. For participants, handedness was not reported in two of the studies. Ninety-two per cent of individuals with RE were righthanded compared to seven per cent who were lefthanded and one per cent who were ambidextrous. In the controls prevalence of handedness was similar, eighty-seven per cent were righthanded, ten per cent were lefthanded, and three per cent were ambidextrous. In four studies controls handedness was not reported.

The main difference between the studies in participant characteristics was the age of the cohorts and sex ratios. Age varied considerably but was often well controlled. Sex ratios, however, varied greatly between the participants and controls.

3.3.4 **Epilepsy characteristics**

Studies	Star Rating	Mean age of seizure onset (years)	Mean duration (months)	Seizure frequency (per year)	AEDs (YES/NO)	Polypharmacy	Spike location Right/Left/Bilateral (emphasis)/None	Seizure remission (YES/NO)
Lin et al (2012)	5	9.5±1.4	6.5±3.8	Not reported	8/5	0	Not reported	No
Overvliet et al (2013)	4	7.3±2.2	28±24	Not reported	16/8	5	Not reported	No
Pardoe et al (2013) (Group A)	6	Not reported	Not reported	Not reported	10/6	Not reported	Not reported	Not reported
Pardoe et al (Group B)	4	Not reported	Not reported	Not reported	3/6	Not reported	Not reported	Remission is quite probable in this group
Pardoe et al (Group C)	4	Not reported	Not reported	Not reported	No	Not reported	Not reported	10
Garcia-Ramos et al (2015).	6	Not reported	6.5±3.4	Not reported	8/5	Not reported	Not reported	None at baseline. 5 at follow-up
Kim et al (2015)	4	6.9±1.7	Not reported	Prior to diagnosis 3.8±1.3	None at time of scanning	None at time of scanning	4/8/8	Not reported
Luo et al (2015)	4	8.02±1.64	13.41±12.96	Not reported	9/12	0	4/7/10	No

Table 13: Epilepsy characteristics. Mean age of seizure onset, duration of epilepsy, seizure frequency, use of AEDs, polypharmacy, spike location, and whether any of the participants had seizure remission (yellow area). The first two columns (coloured in light blue) contain the studies name and quality rating.

Table 13 contains information on the epilepsy characteristics of the groups. The mean age of seizure onset ranged from 6.9±1.7 to 9.5±1.4 years. The mean duration of seizure periods ranged between 4.83±1.09 to 28±24 months. The youngest mean seizure onset was in (Kim *et al.*, 2015) and the oldest was in (Lin *et al.*, 2012) In those studies that reported the mean duration, this ranged from 6.5±3.4 (Garcia-Ramos *et al.*, 2015) to 28±24 months (Overvliet *et al.*, 2013). There was a lack of reporting of the location of epileptiform spikes in participant electroencephalographic (EEG) recordings, six out of the eight groups under-reported. Of those studies that did report, bilateral spikes seemed to be more prevalent (35) compared to the unilateral right (15) or left (20) sided spikes.

Antiepileptic drugs (AEDs) were used in almost half (45.86 %), based on studies where this information was reported. Polypharmacy was underreported, and only five participants (Overvliet *et al.*, 2013) were reported to be treated with multiple AEDs. The reporting of seizure frequency and whether participants were in seizure remission was rare: one study reported on seizure frequency (Kim *et al.*, 2015) and six studies acknowledged seizure remission. Groups of children with seizure remission were seen in follow-up and specific cross-sectional groups. (Pardoe *et al.*, 2013; Garcia-Ramos *et al*., 2015).

Overall, the duration of epilepsy, as expected, was hugely different, varying from recently newly diagnosed to over two years duration. Epilepsy duration did not affect the distribution of spikes, which were fairly similar across the studies. Similarities across studies were seen in the age of epilepsy onset, confirming the typical age of incidence, and limited use of antiepileptic drugs. Polypharmacy was also rare. Most studies acknowledged seizure remission but only a few included information about which participants were in seizure remission.

3.3.5 **Methodologies**

Study	Stars	Longitudinal Study	Structural MRI Protocol. (TR/TE, Flip Angle and Slice thickness)	Magnet Strength (Tesla)	Analysis
Lin et al (2012).	5	No	24/5 ms, 40°, 1.5 mm	1.5	FSL FIRST
Overvliet et al (2013).	4	No	8.3/3.8 ms, not reported, 1 mm	3	Freesurfer
Pardoe et al (2013) (Group A)	6	No	9/1.9 ms, 20°, 2 mm	3	Freesurfer and SPM
Pardoe et al (2013) (Group B)	4	No	1730/4.38 ms, 15°, 1.6 mm	1.5	Same as group A
Pardoe et al (2013) (Group C)	4	No	1730/3.05 ms, 15°, 1.6 mm	1.5	Same as group A
Garcia-Ramos et al (2015).	6	Yes	24/5 ms, 40°, 1.5 mm	1.5	Freesurfer
Kim et al (2015).	4	No	9.8/4.6 ms, 8°, 1mm	3	Freesurfer
Luo et al (2015).	4	No	6.008/1.984 ms, 90°, 1 mm	3	SPM and DARTEL

Table 14: Methodologies and findings in grey mater studies. Included are whether the study is longitudinal, the structural MRI protocol, magnet strength and the type of neuroimaging analysis performed.

Table 14 contains information on the scanning strategies and protocols for each study. The slice thickness of the MR images varied between 1-5 mm. Five 3 Tesla and five 1.5 Tesla scanners were used. Most T1 images were generated by a short time to repetition (TR) and echo sequences except for Pardoe *et al*. study, which at two sites used a long TR of 1730 ms with a short TE of 4.38 ms or less. Volumetric analysis was mainly performed using the voxel-based morphometry (VBM) function of SPM (Ashburner and Friston, 2000) and cortical thickness was predominantly measured using Freesurfer (Dale, Fischl and Sereno, 1999).

3.3.6 **Hypotheses and findings**

Study	Hypothesis (extracted from paper)	Baseline significantly thicker regions	Baseline significantly thinner regions	Follow-up significantly thicker regions	Follow-up significantly thinner regions	Significant grey matter volumetric or shape changes	Disproved null hypothesis
Lin et al (2012).	Compared to controls 1. Investigate neuroanatomic alterations in children with BECTs. 2. Variations in the shape of subcortical structures.	N/A	N/A	N/A	N/A	Putamen volumes larger than controls. Diffuse hypertrophic regions in rostral and caudal putamen. Left caudate expansion in the dorsal and ventral regions.	Nothing to disprove
Overvliet et al (2013).	The goal of the current study is to investigate whether abnormalities in cortical thickness can be found in RE, both within and beyond the sensori-motor cortex. Furthermore we investigated whether such abnormalities are located in the left perisylvian language areas	None	Left hemisphere. Thinner in the supramarginal gyrus and partly covered the bank of the superior temporal sulcus, the superior temporal gyrus and lower post-central gyrus.	N/A	N/A	None reported	Yes, differences were found within and beyond the sensory motor cortex. In addtion these have been found within the left perisylvian language areas.
Pardoe et al (2013) (Group A)	Examine whether any differences observed might progressively vary as a function of age (maturation)	N/A	N/A	N/A	N/A	Increased volume in regions of the bilateral middle frontal gyrus, cingulate and left superior parietal lobe.	If combined with groups B and C then differences are apparent.
Pardoe et al (2013) (Group B)	Same as group A	Bilateral middle and inferior frontal gyri and supramarginal gyrus. Left insular cortex. Scattered regions in the parietal lobes.	Part of the right central sulcus	N/A	N/A	Increased volume in parts of the bilateral middle frontal gyrus	If combined with groups A and C then differences are apparent.
Pardoe et al (2013) (Group C)	Same as group A	No significant differences	No significant differences	N/A	N/A	Increased volume in part of the left middle frontal gyrus	If combined with groups A and B then differences are apparent.
Garcia-Ramos et al (2015).	Investigating differences in cortical thickness and putaminal volume.	None	Bilateral middle frontal gyrus (rostral), left inferior temporal gyrus, left lateral occipital gyrus and right cuneous.	Left rostral middle frontal gyrus, insula, bilateral occipital gyrus, post-central gyrus and right superior frontal gyrus and pre-central gyrus	Left isthmus of the cingulate.	Increased putamen volumes at baseline and follow-up. Putamen decreased in volume over time and this was most pronounced in the right putamina.	Yes, differences were found.
Kim et al (2015).	Case-control study to identify abnormal cortical structures.	Right superior frontal gyrus, right superior and middle temporal gyri and precuneus. Left oribito-frontal gyrus, pars orbitalis gyrus and precentral gyrus.	Nothing reported	N/A	N/A	Larger putamen and amygdala in BECTs.	Unclear
Luo et al (2015).	We hypothesise that the regions with significant changes in GM volume may be involved in functional connectviity abnormalites.	N/A	N/A	N/A	N/A	Increased grey matter volume in the bilateral putamen, and para-central lobule. Right interior insula/frontal operculum, right supplmentary motor area (SMA), right inferior temporal gyrus and left cerebellum.	N/A

Table 15: Detailed overview of the hypotheses and findings of grey matter studies. Included are the study hypotheses and whether the null hypothesis was disproved. Also included are details of significantly thicker or thinner regions or cortical volumes, compared to controls

Hypotheses were not well defined (*Table 15*). However, they loosely focussed on cortical thickness in sensorimotor and perisylvian language areas, volumes and shape differences of subcortical volumes such as caudate, putamen and thalamus and how these relate with age as a regressor or in a longitudinal investigation.

Significantly thicker cortical regions were rare but were found in two studies, (Kim *et al.*, 2015) and (Pardoe *et al*., 2013) (group A). These found thickening in the bilateral middle and inferior frontal gyri and right superior frontal gyrus. There was greater reporting of regions of cortical thinning. Three studies, (Overvliet., *et al.* 2013), (Pardoe *et al.*, 2013) (group B) and (Garcia-Ramos *et al.*, 2015) found regions of cortical thinning. Thinning was seen involved the bilateral middle frontal gyrus and the left superior and inferior temporal gyrus. In the single longitudinal study, a significant difference in thickness at follow-up included the left rostral middle frontal gyrus, insula, bilateral occipital gyrus, post-central gyrus and right superior frontal gyrus and precentral gyrus. The only evidence of thinning was seen in the left isthmus of the cingulate (Garcia-Ramos *et al.*, 2015).

In contrast with the trend for cortical reduction, an increase in grey matter volume was seen predominantly in subcortical structures. Six of the studies (all three groups from the (Pardoe *et al*., 2013) research) showed an increase in grey matter volume in the neocortex. This included (C. Luo *et al.*, 2015), (Kim *et al.*, 2015) and (Garcia-Ramos *et al.*, 2015). In particular putamina, volumes were larger than controls in four studies (Lin *et al.*, 2012; C. Luo *et al.*, 2015; Garcia-Ramos *et al.*, 2015; Kim *et al.*, 2015). Longitudinal measurements (Garcia-Ramos *et al.*, 2015) show that the putamen volume decreases over time but essentially remains enlarged compared to controls. The greatest change in volume occurred in the right putamen. Other enlarged subcortical structures included the bilateral amygdala (volume) (Kim *et al.*, 2015) and the left caudate (shape difference) (Lin *et al.*, 2012).

Increased volume was detected in parts of the bilateral middle frontal gyrus, but this was only reproducible between the groups in the (Pardoe *et al*. 2017) study. In most of the studies, it was unclear whether the generated hypotheses could be answered. The best-designed hypothesis (Overvliet., *et al.*, 2013) demonstrated that reduced cortical thickness was found within and beyond the sensory cortex and perisylvian language areas.

3.3.7 **Analysis**

Study	Left Putamen (Cohens d)	Right Putamen (Cohens d)	Combined Putamen (Cohens d)
Lin et al (2012)	N/A	N/A	0.97
Garcia-Ramos et al (2015)	0.86	0.71	N/A
Kim et al (2015)	0.97	1.13	N/A
Luo et al (2015)	1.13	1.26	N/A
Average	0.99	1.03	0.97

Table 16: Table of putamina effect sizes and the overall average for each study that reported on putamen/putamina volumes. Most of the studies provided the putamen size for each hemisphere. There was one study which reported a combined volume of the left and right putamen.

The most pertinent feature of the grey matter studies was the enlarged subcortical structures; in particular the putamina (Table 16). To calculate the effect size between individuals with RE and controls, the putamen volumes and/or t scores were extracted from the papers. The calculated effect sizes (Cohen's d) show a range in the left putamina between 0.86 and 1.13 and in the right putamina 0.73-1.26. The average for left putamina was 0.99 and for the right putamina a slightly increased effect size of 1.03.

		Increased **thickness**/*volume*				Decreased **thickness**/*volume*			
Study	Mean Age (years)	Frontal lobe	Temporal lobe	Parietal lobe	Occipital lobe	Frontal lobe	Temporal lobe	Parietal lobe	Occipital lobe
Kim et al (2015)	7.5±1.5	**L/R**	**R**	**R**	No	No	No	No	No
Luo et al (2015)	9.12±1.51	*L/R*	*R*	*L/R*	No	No	No	No	No
Pardoe et al (2013) (Group A)	9.3±1.6	*L/R*	No	*L*	No	No	No	No	No
Garcia-Ramos et al (2015)	10.3±1.9	No	No	No	No	**L/R**	**L**	**R**	**L**
Overvliet et al (2013)	11.3±1.9	No	No	No	No	No	**L**	**L**	No
Pardoe et al (2013) (Group B)	15.8±2.3	**L/R**	*L*	*L/R*	No	***R***	No	**R**	No
Pardoe et al (2013) (Group C)	22.7±2.7	*L*	No	No	No	No	No	No	No

*Table 17: Grey matter studies with cortical regions significantly different than controls stratified by group mean age. Differences include increased or decreased (**bold**) thickness and increased or decreased (italics) volume. Included are brain regions and the laterality of the effect. Thicker or increased grey matter volume (yellow). Distribution: L=Left and R=Right. Those regions which showed no difference (dark blue).*

		Increased **thickness**/*volume*				Decreased **thickness**/*volume*			
Study	Mean epilepsy duration (months)	Frontal lobe	Temporal lobe	Parietal lobe	Occipital lobe	Frontal lobe	Temporal lobe	Parietal lobe	Occipital lobe
Pardoe et al (2013) (Group A)	<5 months	*L/R*	No	*L*	No	No	No	No	No
Kim et al (2015)	6±0.5	**L/R**	**R**	**R**	No	No	No	No	No
Garcia-Ramos et al (2015)	6.5±3.4	No	No	No	No	**L/R**	**L**	**R**	**L**
Luo et al (2015)	13.41±12.9	*L/R*	*R*	*L/R*	No	No	No	No	No
Overvliet et al (2013)	28.8±0.16	No	No	No	No	No	**L**	**L**	No

*Table 18: Grey matter studies with cortical regions significantly different than controls stratified by mean duration of epilepsy. Differences include increased or decreased thickness (**bold**) and increased or decreased volume (italics). Included are brain regions and laterality of the effect. Thicker or*

increased grey matter volume (yellow). Distribution: B=Bilateral, L=Left and R=Right. Those regions which showed no difference (dark blue). Studies are ordered by the mean duration of epilepsy.

	Thicker Cortex				Thinner Cortex			
	Frontal lobe	Temporal lobe	Parietal lobe	Occipital lobe	Frontal lobe	Temporal lobe	Parietal lobe	Occipital lobe
Baseline (Age 10.3±1.9)	No	No	No	No	L/R	L	R	L
Follow-up (2 years later)	R	L	No	L/R	No	No	No	No

Table 19: Statistically significant difference in cortical thickness and thinness in Garcia-Ramos et al. at baseline and two years follow up. The table is divided into thicker and thinner. Included are brain regions and laterality of the effect. Thicker or increased grey matter volume (yellow). Distribution: B=Bilateral, L=Left and R=Right. Those regions which showed no difference (dark blue).

The analysis was simplified by sorting the data into their cortical lobe location and coding whether the measurements were volume or thickness or both. Also, the findings were stratified by the mean age of the participants (*Table 17*).and the duration of epilepsy to see which factors had a greater impact on cortical features. A similar analysis was performed on the sole longitudinal study (Garcia-Ramos *et al.*, 2015).

Cortical differences with healthy controls were apparent in all studies within a broad age range (7-22 years). Moreover, there appeared to be a difference between cortical thickness in younger children (<10 years) and older children (>10 years). In younger children, *thicker* cortical regions were more apparent (Pardoe *et al.*, 2013 (Group A); Kim *et al.*, 2015; Luo *et al.*, 2015). Regions of thicker cortex were reported in the bilateral frontal, right temporal and parietal lobes. In older children, *decreased* cortical thickness was more apparent (Garcia-Ramos et al., Overvliet et al. and Pardoe et al. (Group B)). Regions of thinner cortex were seen predominantly in the parietal lobe (3 studies, right-sided emphasis). The bilateral and left frontal (two studies), left temporal (two studies) and left occipital (one study) lobes were all identified to be thicker in older children.

Similarly, increased cortical volumes were more apparent in younger children compared to older. In younger children, the bilateral frontal (two studies), right temporal (one study) and bilateral and left parietal lobes (two studies) showed regions of increased volume. Increased grey matter volume was less prevalent in adolescents and adults compared to children with RE. Areas with increased volume included the bilateral and left frontal (two studies), left temporal (one study) and the bilateral parietal lobe (one study). Only one study demonstrated a decrease in volume, and this was in an adolescent group, Pardoe *et al*. (Group B). The region affected was contained within the right frontal lobe.

Table 8 demonstrates what association epilepsy duration has on the distribution of cortical differences. An epilepsy duration of less than a year showed both cortical thickening and thinning. The Garcia-Ramos *et al*. study demonstrated thinner cortex and did not fit the trend for a thicker cortex. In studies where the participants had a duration of epilepsy over a year, they also demonstrated mixed results with both evidence of increased and decreased thickness. The Overvliet *et al.* study had the longest epilepsy durations of 28.8±0.16 months. This study demonstrated the least amount of regional differences with only the left temporal and parietal lobes demonstrating increased cortical thickness. There is only one longitudinal study in this review (Garcia-Ramos *et al.*,

2015). This study looked at changes in cortical thickness over time. It demonstrated at baseline regions of thinner cortex in the bilateral frontal lobe, left temporal and occipital and the right temporal lobes compared to controls. In the follow-up scans (2 years later) the cortex was thicker in the right frontal lobe, left temporal lobe and bilateral occipital lobes.

Overall, significant differences in cortical thickness/volume were seen across the age ranges. In particular, the cross-sectional studies showed increased cortical volume and thickness in younger children (<10 years) whereas in older children (>10 years) thinner cortical regions and smaller volumes are the most apparent. The single longitudinal study suggests thickening of cortical regions in late childhood to early adolescence. The longitudinal findings further add to the evidence that both regions of increased and decreased cortical thickness/volumes are apparent in adolescence when the likelihood of seizure remission is high. The association of epilepsy duration on brain structure is unclear; however, it appears that in children with a longer duration of epilepsy (over two years) there are fewer brain changes in both cortical thickness and volume compared to children with new-onset RE.

3.4 White matter studies

3.4.1 Quality check

Paper	Is the case definition adequate?	Representatives of the cases	Selection of controls	Definition of controls	Comparability of cases and controls on the basis of design and analysis	Stars (out of 6)
Besseling et al (2013)	1 Star: Independent validation. Based on clinical and EEG data	Recruited from a specialised epilepsy centre. Excluded children with dyslexia	No description	1 Star: Healthy controls	2 stars: Age and gender as covariates	4
Ciumas et al (2014)	1 Star: Independent validation. Based on clinical and EEG data	1 Star: Representative from a paediatric epilepsy clinic.	Healthy volunteers. No details of recruitment	1 Star: Healthy controls	2 Stars: Age and gender as covariates	5
Kim et al (2014)	1 Star: Independent validation. Based on clinical and EEG data	Recruited from a paediatric epilepsy clinic. Excluded children with developmental disabilites. Included children with bilateral tonic seziures.	1 Star: Community controls. Close friend of patients. Similar age, gender, socio-economic status and educational level	1 Star: Healthy controls	2 stars: Age and gender as covariates	5
Xiao et al (2014)	1 Star: Independent validation. Diagnosed by the International League Against Epilepsy Criteria.	Recruited from West China Hospital of Sichuan University (one patient had myoclonic seizures, four with very high seizure frequencies)	Healthy volunteers. No details of recruitment.	1 Star: Healthy controls.	1 Star: Age as a covariate.	3
Wu et al (2015)	1 Star: Independent validation. Diagnosed by the International League Against Epilepsy Criteria.	Recruited from Beijing Childrens Hospital. Patients <7 and >14 years excluded.	No description of how the controls were obtained.	1 Star: Healthy Controls	No evidence	2

Table 20: White matter quality check results in date order. Sections: Yellow criteria fulfilled (1 Star); white criteria unfulfilled. Articles >3 stars= blue, <3 stars = red

The results of the white matter study Newcastle-Ottawa quality check are presented above (Table 20). None of the studies obtained a full score. The range of quality was 2-5 stars. The mean quality score was 3.8±0.7 stars. Quality was lost in obtaining representative cases and the under-reporting of control recruitment. One study (Wu *et al.*, 2015) scored below average quality and subsequently was excluded from further analysis.

3.4.2 **Participant characteristics**

Studies	Stars	Number of RE	Number of controls	RE Mean Age (years)	Control Mean Age (Years)	RE Male	Controls Male	RE Female	Controls Female	RE and Control Male ratio	RE Handedness (Right/Left/Ambidextrous)	Control Handedness (Right/Left/Ambidextrous)
Besseling et al (2013)	4	23	23	11.4±2 years	10.4±1.6	14	12	9	11	0.64	19/3/1	12/2/0
Ciumas et al (2014)	5	25	25	9.6±1.9	10±3	18	14	7	11	0.39	20/3/2/	19/5/1
Kim et al (2014)	5	19	25	10.7±2.4	10.4±2.7	11	14	8	11	0.73	Not reported	Not reported
Xiao et al (2014)	3	28	18	9.4±2.3	10.1±1.9	14	11	14	7	1.00	28/0/0	18/0/0

Table 21: Participant characteristics in white matter neuroimaging studies. Included are characteristics of participants with RE and Controls. Mean age (years), sex and sex ratios and handedness.

3.4.3 **Epilepsy characteristics**

Studies	Stars	Age of seizure onset (years)	Duration of epilepsy (months)	Seizure frequency (per year)	AEDs (YES/NO)	Polypharmacy	Spike location Right/Left/Bilateral (emphasis)/None	Seizure remission (YES/NO)
Besseling et al (2013)	4	7.5±2.1	46.8±24.08	Not reported.	13/10	4	Not reported	Not reported
Ciumas et al (2014)	5	8.38±2.14	20.7±20	8.1	10/18	2	2/14/9/0	Not reported
Kim et al (2014)	5	Not reported	36.39±18	Not reported.	14/5	0	4/6/9/0	Three participants are probably in remission
Xiao et al (2014) (Group A)	3	8.15±2.2	Not reported	At least 2 seizures in the past year	10/5	2	15/0/0/0	No
Xiao et al (2014) (Group B)	3	8.87±2.35	Not reported	At least 2 seizures in the past year	10/3	1	0/13/0/0	No

Table 22: Participant epilepsy characteristics in white matter studies. Information is provided for the age of seizure onset, duration of epilepsy, seizure frequency, the prevalence of AED usage and polypharmacy (>1 AED). Furthermore, the location of spikes and whether any of the participants were in remission was included. Note that the Xiao et al. study has been divided into two groups, Group A: Right-sided spikes, Group B: Left-sided spikes.

The details of participant characteristics are included in

Table 21. Ninety-five children with rolandic epilepsy were scanned over four studies; the number of controls (91) was similar. Their mean age ranged from 9.4±2.3 to 11.4±2 years, which was wider than the age range of controls (10±1.5 years to 10.4±2.7). In total, 57 males and 38 females with RE were scanned. Sex ratios, on the whole, were kept balanced however there was one study (Ciumas *et al.*, 2014) where there were far more male participants than female (F: M ratio 0.39). Handedness was mostly reported, eighty-six per cent were righthanded, twelve per cent were lefthanded, and two per cent were ambidextrous. The distribution of handedness was roughly similar to the handedness of the controls; eighty-eight per cent were righthanded, eleven per cent were lefthanded, and one per cent was ambidextrous. Overall, most characteristics were fairly similar across the studies. Differences were seen in the sex ratios and to a lesser extent the age ranges of the children with RE

The age of seizure onset was fairly similar across the studies (Table 22). The duration of epilepsy, however, was on the whole quite long. The longest mean duration was 46.8 months. Seizure frequency information was limited or unreported with only one paper (Ciumas *et al.*, 2014), providing accurate reports. Most studies had a similar ratio of medicated to un-medicated participants. Nine of the participants across the studies were classified as polypharmacy with the highest incidence in (Besseling, *et al.*, 2013). There was mixed reporting of spike location, 42 had the right side, 33 had left-sided, and 18 had bilateral. Remission was frequently under-reported in the literature; it is suspected from the age of those scanned that only three of the participants across the studies were in remission (Kim *et al.*, 2014). Overall, there are many similarities between the studies. Nevertheless, the main differences between the studies were the duration of epilepsy and the use of polypharmacy.

3.4.4 **Methodologies**

Study	Stars	Structural MRI Protocol (TE/TR, Flip angle, slice thickness)	DWI Protocol (TE/TR, Gradient directions, DWI B-values, Number of B0 images)	Magnet Strength (Tesla)	Methodology for analysing diffusion weighted images.
Besseling et al (2013)	4	8.3/3.8 ms, No flip angle reported, 1 mm.	72/6600 ms, 66, 1200 s/mm², 1	3T	Tract based spatial statistics
Ciumas et al (2014)	5	2400/3.55 ms, 8°, 1.6 mm.	86/6900 ms, 48, 10,000 s/mm², 4	1.5 T	Voxel based analysis
Kim et al (2014).	5	1160/4.19 ms, 15°, 1.2 mm	Not reported, 15, 1000 s/mm², not reported	3T	Tract based spatial statistics
Xiao et al (2014)	3	Not acquired	93/6800 ms, 20, B-value 1000s/mm², not reported	3T	Tract based spatial statistics

Table 23: Methodologies of white matter studies. Included are the T1 structural and diffusion-weighted image protocols with scanner magnet strength and neuroimaging analysis protocols.

The majority of the studies were performed on 3T systems (Table 23). A large range of gradient directions was used (15-66), in contrast, B-values were fairly similar (1000-1200s/mm²) there was one stand-out b-value of 10,000 (Ciumas *et al.*, 2014), this is believed to be a typographical error, but we are still awaiting confirmation (Ciumas *et al.*, 2014). The majority of the studies used the FSL tracts based spatial statistics (TBSS) to analyse the data; only one paper (Ciumas *et al.*, 2014) used voxel-based analysis. The acquisition of structural T1 MR images for usage as a template is not necessary for DWI imaging analysis. As a result their use was mixed, one study used a T1 weighted image (Besseling, *et al.*, 2013) with 1 mm slice thickness, whereas two utilised images with greater T2 weighting (Ciumas *et al.*, 2014; Kim *et al.*, 2014) and one did not acquire any (Xiao *et al.*, 2014) and relied on Montreal Neurological Image (MNI) space templates.

3.4.5 **Hypothesis and findings**

Study	Hypothesis	Group MD values (Regions or tracts)	Group FA findings (Regions or tracts)	Disproved null hypothesis
Besseling et al (2013)	In children with RE white matter tracts connecting to rolandic regions may be compromised.	Not reported	Tractography: Lower FA in streamlines between the bilateral pre and post central gyri and the, ipsilateral insula and superior temporal gyrus. Bilateral pre-central gyri and the superior frontal cortex and the par opercularis. Left precentral gyrus and caudal mid frontal cortex and pars opercularis. Bi-lateral post-central gyri and supramarginal gyri. Local connections between bilateral pre and post-central gyri. TBSS: No significant difference in FA.	Unclear. The team do not define what compromised means.
Ciumas et al (2014)	In children with RE, the age dependence of the epilepsy and cognitive abnormality suggest the possibility of altered maturation of white matter.	Higher MD, left, post-central gyrus, cuneus, middle frontal gyrus and inferior parietal lobe. Right post-central gyrus, medial frontal gyrus, cuneus (two clusters).	Lower FA in the left precentral and post central gyrus.	Unclear. Measurements of FA and MD appeared to affected by the duration of the epilepsy.
Kim et al (2014).	Alterations in brain in white matter microstructure in certain brain areas would correlate with specific cognitive impairments in RE.	Higher MD in the left superior longitudinal fasiculus (three clusters), retrolenticular part of internal capsule, posterior thalamic radiation and saggital striatum.	No significant difference in FA.	Yes, measurements of white matter changes (increased axial diffusivity) in the left hemisphere correlates with lower verbal IQ.
Xiao et al (2014) (Group A)	White matter integrity is comporomised in children with active BECTs those with epileptic foci on the dominant hemisphere for language were more vulnerable to damage from seizures.	Increase in MD in the right inferior fronto-occipital fasciculus, anterior thalamic radiation and left superior lonigtudinal fasciculus and cingulate gyrus.	Decreased FA in the left superior longitudinal fasciculus, cortical spinal tract, cingulum, anterior thalamic radiation and inferior fronto-occipital fasciculus.	Unclear, white matter changes are seen in children with right sided (non dominant hemisphere) spikes however it is unknown whether this equates to damage.
Xiao et al (2014) (Group B)	White matter integrity is comporomised in children with active RE and those with epileptic foci on the dominant hemisphere for language were more vulnerable to damage from seizures.	Increased MD in body and splenium of the corpus callosum, bilateral superior lonigtudinal fasiculus, cingulate gyrus and corticospinal tract.	Bilateral reduced FA found in body, splenium of the corpus callosum, forceps minor and major, superior longitudinal fasciculus, cingulate gyrus, anterior thalamic radiation. Right inferior fronto-occipital fasciculus and cortical spinal tract.	Unclear, greater white matter changes are seen in children with Lt sided spikes and RE. However, differences in white matter FA and MD do not equate to damage.

Table 24: Detailed overview of the hypotheses and findings of white matter studies.

Included are the hypothesis and whether the null hypothesis was disproved. Furthermore, included are the regions of statistically significant difference for MD and FA.

Hypotheses were non-specific and related to the maturity of white matter, cognition and “damage” from seizures and spikes. Nearly all studies reported values of mean diffusivity (MD) and fractional anisotropy (FA) except for one (Besseling *et al.*, 2013), which neglected to report MD values. Half of the studies reported findings for specific anatomical tracts (Besseling *et al.*, 2013; Kim *et al.*, 2014; Xiao *et al.*, 2014), one reported on streamlines between brain regions (Besseling *et al.*, 2013) and the rest for brain regions (Besseling *et al.*, 2013; Ciumas *et al.*, 2014). There was one point of agreement between the studies, which was a higher MD in the left superior longitudinal fasciculus. Fractional anisotropy (FA) was reported in all studies but was able to detect differences between groups in all but two studies. This made interpretation difficult. Although there were these problems, the only reproducible finding was a measure of reduced FA in the left superior longitudinal fasciculus and the anterior thalamic radiation and within the left pre and post-central gyri.

In summary, changes in MD and FA can be detected in many different white matter tracts in children with RE, predominantly in the left hemisphere. Both MD and FA appear to be a good measure of differences in white matter microstructure, but this is not consistent. It appears that left hemisphere structures are the most affected in particular the superior longitudinal fasciculus and anterior thalamic radiation and the pre and post-central gyri.

3.4.6 **Analysis**

A simplification of the FA and MD data for tracts derived from TBSS are represented in Table 25. TBSS was used in three studies (four groups) (Besseling., *et al.*, 2013; Kim *et al.*, 2014; Xiao *et al.*, 2014) and was the only similarity in methodologies across the groups. These data were stratified by mean age (Table 25) at the scan and mean duration of epilepsy (Table 26).

Study	Mean Age	Cingulum	Corpus Callosum	Corticospinal tract	External capsule	Fornix	Inferior network	Internal capsule and thalamic radiations	Mid-brain	Peri-sylvian networks
Xiao et al (2014) (Group A)	8.87±2.23	***L***	No	*L*	No	No	***L*/R**	***L*/R**	No	***L***
Xiao et al (2014) (Group B)	10.03±2.45	***L/R***	***L/R***	**L**/*R*	No	No	*R*	*L/R*	No	***L*/R**
Kim et al (2014).	10.7±2.4	No	No	No	No	No	No	**L**	No	**L**
Besseling et al (2013)	11.4±2	No	No	No	No	No	No	No	No	No

Table 25: Findings of TBSS in white matter studies stratified by respect to mean age. . Measurements of FA and MD are presented with the distribution of the affected white matter structures. L- Left and R-Right. **Mean diffusivity (BOLD)** *Fractional anisotropy (italics). Yellow squares- a significant difference. Blue squares-no significant difference. The JHU white matter tract regions are on the top row, and they have been grouped to ease understanding.*

Study	Epilepsy duration (years)	Cingulum	Corpus Callosum	Corticospinal tract	External capsule	Fornix	Inferior network	Internal capsule and thalamic radiations	Mid-brain	Peri-sylvian networks
Xiao et al (2014) (Group B)	1.16±0.10	***L/R***	***L/R***	**L**/*R*	No	No	*R*	*L/R*	No	*L/R*
Xiao et al (2014) (Group A)	1.26±0.5	***L***	No	*L*	No	No	*L*/**R**	*L*/**R**	No	***L***
Kim et al (2014).	3.3±1.5	No	No	No	No	No	No	**L**	No	**L**
Besseling et al (2013)	3.9±2.1	No	No	No	No	No	No	No	No	No

Table 26: Findings of TBSS in white matter studies ordered by epilepsy duration. Measurements of FA and/or MD are presented with the distribution of the affected white matter structures. B- Bilateral, L- Left and R-Right. **Mean diffusivity (BOLD)** *Fractional anisotropy (italics). Yellow squares- significant difference. Blue squares-no significant difference. The JHU white matter tract regions are on the top row and have been grouped together for ease of understanding.*

Across all of the studies, the external capsule, fornix, and midbrain white matter structures are similar to healthy controls (Table 25). Despite this, differences in FA and MD can be found in the cingulum, corpus callosum, corticospinal tract, inferior network, internal capsule and thalamic radiations and peri-Sylvian networks. However, there is a lack of congruence between the studies except for findings of decreased FA and increased MD in the internal capsule and thalamic radiations and peri-Sylvian networks (with a left hemisphere emphasis). This relative congruence in the tracts, as mentioned above, was detected between the ages of 8.87±2.23 and 10.7±2.4 years. Most bilateral changes were seen in (Xiao *et al.*, 2014) Group B. This group had a mean age of 10.03±2.45 and contained children with only left-sided spikes on their EEG. Nevertheless, in the same age group and older (Besseling *et al.*, 2013; Kim *et al.*, 2014), there were few changes in white matter tracts. Table 16 demonstrates white matter differences in relation to the duration of epilepsy. In studies where seizures have been present for one year, there is evidence of multiple differences in white matter structures, which are predominantly bilateral (Xiao *et al.*, 2014). In the RE group, three years after the onset of seizures, the number of white matter changes are minimal and only involve the internal capsule and peri-Sylvian networks (Kim *et al.*, 2014). Close to four years epilepsy duration, there are zero differences in FA and MD between the groups (Besseling *et al.*, 2013).

Overall, it appears that many large white matter structures are affected in children with RE. These differences (mostly in MD) are predominantly in large white matter tracts that stretch within and between hemispheres. Smaller white matter structures and those in the midbrain appear to be untouched. Differences with healthy controls are seen in studies where the patients are close to the onset of seizures and in younger children. These changes are predominantly bilateral (right-sided emphasis). In children who have had a longer duration of epilepsy, the differences with controls are minimal or even non-existent.

3.5 Discussion

3.5.1 Summary of key findings

Nine studies exploring grey matter and five studies exploring white matter were identified which were produced between 1993 and 2017. Grey matter changes occur in children with RE. These changes are diffuse and involve regions inside and outside the rolandic fissure (central sulcus), predominantly within the bilateral frontal and parietal lobes. Additionally, striatal subcortical structures such as putamen, caudate and amygdala are often enlarged and have regional hypertrophic changes in shape. White matter studies also show extensive changes that mainly involve the left superior longitudinal fasciculus and anterior thalamic radiation tracts and connections between the left pre and post-central gyri.

3.5.2 **Grey matter studies**

The grey matter findings suggest a disorder of neurodevelopment in children with RE. The analysis, stratified by age, reveals regions of excessive volume/thickening in younger children, while in older children there are often regions of excessive thinning. The changes are diffuse and include changes both inside and outside the central sulcus as well as striatal subcortical structures. These changes are apparent in cross-sectional studies during the active phase of epilepsy, and thus, it is not possible to conclude a trajectory of early cortical thickness progressing to late cortical thinning. A sole longitudinal study shows findings incongruent with the cross-sectional studies with regions of thinner cortex at baseline. After two years, this changes to sparse patches of predominantly thicker cortex within the regions that were originally thin (Garcia-Ramo*s et al.*, 2015). Despite this incongruence, they identify changes in cortical thickness over time in the active phase of epilepsy. What is still unknown are the transformations in cortical thickness and volume that occur between active epilepsy and seizure remission. Furthermore, are the changes related to the duration of epilepsy, maturation of the individual or a separate seizure remission process? The only evidence of cortical thickness and volume in seizure remission is derived from a single cross-sectional study which demonstrated a near "normalisation" of cortical thickness (Pard*oe et al.*, 2013).

Analysis of cortical features concerning age suggests that there is aberrant cortical development; in particular, a delay to normal cortical thinning. Based on several large longitudinal and cross-sectional studies of normal childhood cortical development, it is the current view that global thinning of the cortex is the predominant process (Walhovd *et al.*, 2017). This trajectory has been seen in children from three years up to young adults (Brown *et al.*, 2012). It is proposed that cortical thickness is a determined by the number of cells within a cortical column and their connections (Rakic, 1995) and the process of global cortical thinning is said to predominantly represent a reduction in synaptic density and the resulting loss of dendritic connections (Stiles and Jernigan, 2010). Cortical volume is a more complex value and represents a product of cortical thickness and surface area (Panizzon *et al.*, 2009). It is likely to be influenced by numerous characteristics of the underlying neural architecture, such as the number of cortical columns and cells (Rakic, 1995; Panizzon *et al.*, 2009). An example of these trajectories is seen in *Figure 12*. Cortical thickness and volume both decrease

with age, thinning is linear, and a decrease in volume is curvilinear. The findings from children with RE suggests a regional deviation from normal cortical developmental trajectories.

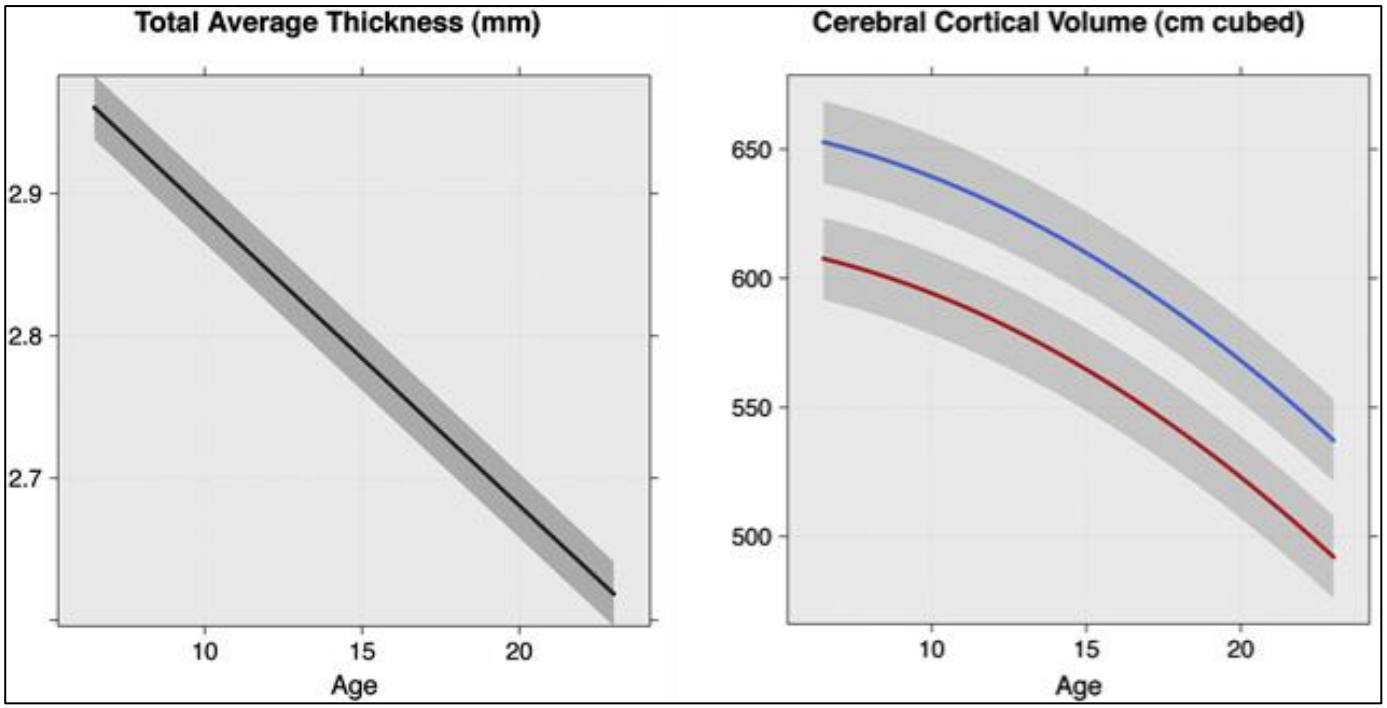


Figure 12: The developmental trajectories of average cortical thickness (in mm), and overall cortical volume (in cm3). The cortical volume trajectories are shown in blue for males and in red for females. Extract from (L. M. Wierenga *et al.*, 2014)*. This figure demonstrates a monotonic decrease in average cortical thickness with age between childhood and adolescence. Whereas with cortical volume, there is a non-linear decrease in this measure during the same period of time.*

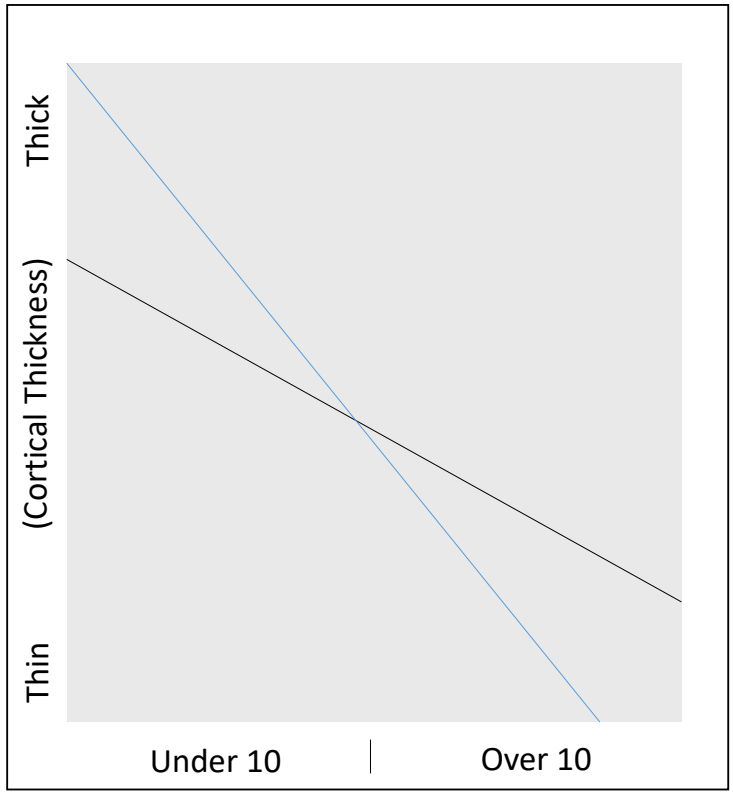


Figure 13: Hypothetical trajectories of global cortical thinning. Healthy children (black line) and abnormal regional cortical thinning children with RE (blue line) based on the evidence from cross-sectional studies. Linear trajectories of global cortical thinning based on (L. M. Wierenga *et al.*, 2014)

A thicker cortex in younger children (<10 years) with RE suggests either a delay to the normal (thinning) development/process, an overgrowth of cortical tissue during early developmental processes or an underdevelopment of the white matter at the cortical border making grey matter appear thicker through the partial volume effect (Sowell *et al.*, 2004). In older children (>10 years), regions of thinning are more apparent. The difference in thickness and volume between younger and older children with RE raises two possibilities. One, a different neurodevelopmental trajectory, such as speeded up cortical development with over-pruning or two, potential damage to the cortex due to inter-ictal discharges or seizures.

Figure 13 demonstrates the theoretical trajectories of cortical thinning in healthy children and those with RE based on the cross-sectional data. It is important to note that these trajectories assume that in regions of difference, the cortical thinning is a linear process. Furthermore, this hypothesis does not fit with the longitudinal data, which also suggests a delay in thinning in cortical regions in initially thinner regions. Nevertheless, there is one point of accord between cross-sectional and longitudinal studies, the evidence of possible delayed cortical thinning in the under 10s (thicker cortical regions) and the thickening (delayed thinning) of cortical regions in the longitudinal study. All studies have demonstrated that differences in cortical thickness and volume in children with RE are patchy and regionalised. The distribution of these regional changes appears to be different with age in children with RE.

In younger children, the regions of increased thickness were more likely to be bilateral with a right hemisphere predominance (Cheng Luo *et al.*, 2015; Kim *et al.*, 2015) whereas in older children the cortical thinning was seen predominantly over the left hemisphere. Any cortical thickening in older children also appeared to have a left-sided tendency (Overvliet, *et al.*, 2013; Garcia-Ramos *et al.*, 2015). This is a surprising finding as there is evidence in the literature that the greatest cortical thinning in the left dorsal frontal and parietal regions has been seen in children with the greatest gain in verbal intelligence (Sowell *et al.*, 2004). Despite the difference in distribution, regions of the frontal and parietal lobes were more likely to show differences in cortex thickness/volume at any age. Interestingly, these regions were mostly outside of central sulcus and pre and post-central gyrus involving the superior, middle, inferior and orbitofrontal gyrus and the precuneus, cuneus and supramarginal gyrus. It is important to note, that even though there was a trend for cortical thinning in

the over 10s some studies showed increased thickness/volume. These findings, however, were obtained from adolescent groups (mean age 15 and over) where participants were close to or in seizure remission (Pardoe *et al.*, 2013). Features of aberrant development were not only restricted to the cortical regions.

There is substantial evidence of increased volume of the subcortical putamina in children with RE. Enlarged putamen was seen in a group of children around 7-10 years old. In one of the studies, a follow-up, where 62.5 % of participants were in remission, there was a decrease in the volume of both putamina over two years, despite this, a significant difference with controls remained. These volume differences were bilateral, which is in contrast to the cortical data. These findings suggest that the volume of the putamen could be a marker of aberrant brain development, which is useful as the putamen is a relatively well-delineated structure within the subcortical white matter which can easily be measured using volumetric analysis. As group mean putamina volume decreased with an increase in the proportion of participants in remission, there is a suggestion that it may have some association with the remission mechanism. Overall, there appears to be a strong deviation of normal growth trajectories in the putamen in RE. With the addition of those areas of difference in the cortical hemispheres, it may provide insight into the co-occurring cognitive problems.

Increased cortical thickness and or volume in regions of the frontal lobe can be seen in other neurodevelopmental disorders. These include specific language impairment (SLI) (Badcock *et al.*, 2012), developmental coordination disorder (DCD) attention deficit hyperactivity disorder (ADHD) (Langevin *et al.*, 2015) and autism spectrum disorders (ASD) (Wallace *et al.*, 2010; Khundrakpam *et al.*, 2017). A limited number of longitudinal studies have demonstrated possible cortical overgrowth in the frontal lobes in ASD and ADHD during early childhood (Schumann *et al.*, 2010); evidence of accelerated thinning in regions in bilateral parietal, occipital, left frontal and right temporal regions in ASD (Zielinski *et al.*, 2014); and decelerated maturation of the prefrontal regions in ADHD during childhood (Shaw *et al.*, 2007). Similarly, subcortical structural differences in RE can also be seen in other neurodevelopmental disorders.

Striatal structures can also be increased in volume in neurodevelopmental disorders. Reports of increased volume in striatal structures include; the left putamen of children with ASD (Foster *et al.*, 2015) and left or right putamen in speech and language disorders (Liégeois, Mayes and Morgan,

2014) with similar effect sizes to those found in this review. Due to a lack of longitudinal studies on the putamen in neurodevelopment, it is unclear how this structure changes with ageing.

The findings of similar altered brain structure in neuro-developmental disorders further add to the evidence that RE is developmental. It is unclear, though, to what extent the inter-ictal activity and seizures have on these structures. A secondary analysis was performed where the findings were stratified by the duration of epilepsy, to gain insight into this on brain structure.

The secondary analysis did not demonstrate any clear association with epilepsy duration. The findings in children close to the onset of epilepsy (~12 months) reveal many differences with controls, but there is no clear pattern. Some clarity was achieved when groups with the shortest and longest epilepsy duration were inspected. Increased thickness and volume of regions within the bilateral frontal and left parietal lobe in a group (Pardoe *et al.*, 2013) close to onset (<5 months) may suggest that these regions are implicated in the generation of the seizure disorder. In contrast, the group (Overvliet *, et al.*, 2013) with the longest epilepsy duration (28.8±1.8 months) revealed a limited amount of decreased cortical thickness which was lateralised to the left hemisphere and involved the temporal and parietal lobes. These features would suggest that the majority of structural cortical development is normal even after many years of epilepsy. The resulting small regions of difference may reflect an area which has been of affected by the generation of inter-ictal spikes or ictogenesis. Compared to the insight from age stratification, a broad appreciation of these data derived from duration of epilepsy would suggest that the age of the child has a greater impact on the grey matter differences compared to the duration of epilepsy. The changes that occur between active epilepsy and seizure remission would have been useful in understanding the effects of epilepsy; unfortunately, this evidence is limited.

The current neuroimaging evidence suggests that the development of children with RE leads to a reduction in the number of regional differences with healthy children. Nevertheless, regions of abnormal cortical thickness and volume are still apparent in adulthood. This interpretation is only based on one longitudinal and one cross-sectional study. In the longitudinal study (Garcia-Ramos *et al.*, 2015), where under half were in seizure remission at follow-up, there was a demonstration of thicker cortex in frontal, temporal and bilateral occipital regions (Figure 14). Primarily these regions of thickening were similar to those where thinner cortex was found at baseline, but in contrast to the

baseline, these regions were generally smaller. Also, there is also evidence of complete regional cortical normalisation, for example, in the right inferior frontal gyrus.

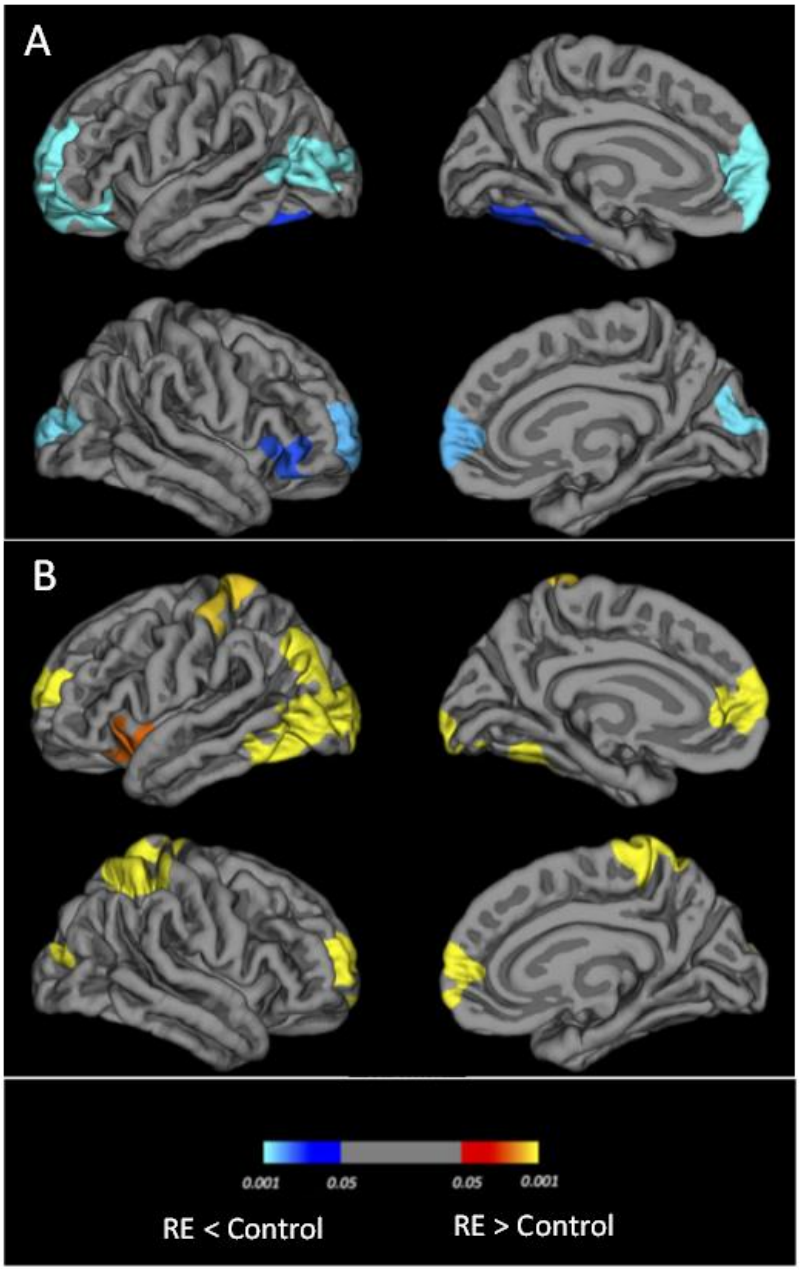


Figure 14: Cross-sectional differences in cortical thickness at baseline and follow-up. Cortical thickness differences with healthy controls at baseline (A) and areas of reduced cortical thinning in follow-up after two years (B). Left hemispheres internal and external are located in the upper portion of images A and B. Right hemispheres internal and external are located below. The colour of the scale bar indicates the statistical significance of the clusters corrected for multiple comparisons (p = <0.05) and age and sex. The left side of the bar, cortical regions in individuals with RE are thinner than controls. Right side of the bar,

cortical regions are thicker in individuals with RE than controls. These images demonstrate brain regions with thinner cortex at baseline which then becomes thicker than controls in the follow-up. Adapted from (Garcia-Ramos et al., 2015)

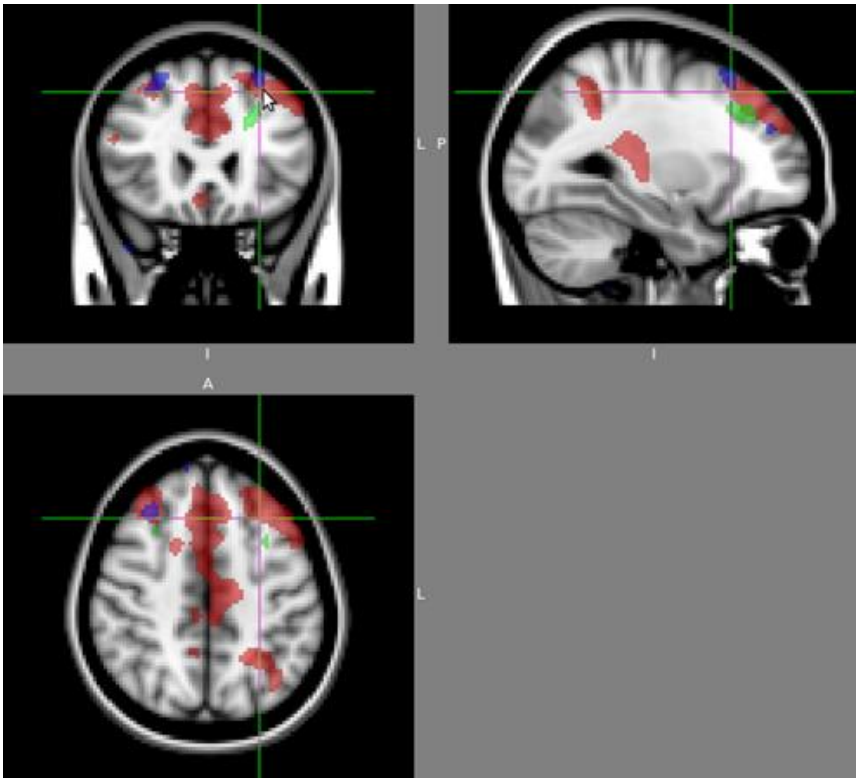


Figure 15: Increased regions of cortical volume in children with RE from different age groups. T1 MRI brain slices (radiological convention). Clockwise from top left; coronal, sagittal and axial slices. Clusters indicate increases in regional volume in children with RE compared to controls. Group A, age 9.3±1.6 (red), Group B, age 15.8±2.3 (blue) and Group C age 22.7±2.7 (green). Extract from supplementary material (Pardoe *et al.*, 2013)

Furthermore, both putamina had decreased in size but remained enlarged compared to controls (Garcia-Ramos *et al.*, 2015), which suggests a persisting disorder of structural neurodevelopment in the putamen, making the putamen a better marker of aberrant development in children with RE. There is also the intriguing possibility that the group reduction in the volume of the putamen is influenced by the fraction of children who are in seizure remission.

In the only cross-sectional study of a group in seizure remission, the only difference with controls was an increased cortical volume of a small region of the left middle frontal gyrus (Pardoe *et al.*, 2013) Figure 15. The persistence of a small region of increased cortical volume in a group of adults in seizure remission from rolandic epilepsy is of great interest. These findings suggest two outcomes; one, that normalisation of the cortex is the predominant process with seizure remission and two, structural differences can persist into adulthood despite seizure remission. The Pardoe findings are of interest as recent research in our lab has found resting-state fMRI connectivity of the left inferior frontal gyrus and superior frontal gyrus to be decreased within children with RE compared to controls during active epilepsy (McGinnity *et al.*, 2017). This overlap between the abnormal structure in remission and differing fMRI connectivity in active epilepsy suggests that in children with RE some cortical differences may persist within the left frontal lobe in seizure remission.

The differences between children with RE and healthy controls involve regional differences of cortical thickness relative to healthy controls predominantly in the frontal and parietal lobes. The thickness of the cortex and its distribution appears to be influenced by the age of the individual rather than the duration of epilepsy, with children under 10 demonstrating thicker cortex and children over 10 demonstrating thinner cortex compared to controls. In addition, the putamen appears to be enlarged, and its volume may be associated with seizure remission. Seizure remission also appears to be associated with normalisation of most of the cortex; however, regions of difference in the left frontal lobe can persist.

These findings suggest a subtle disorder of cortical development with many parallels with other non-lesional neurodevelopmental disorders such as autism, dyslexia, ADHD, specific language impairment and DCD. It would appear that the disorder to the cortical structure is more widespread than the central sulcus and the pre and postcentral gyrus, affecting the frontal and parietal lobes predominantly outside the aforementioned regions and mostly sparing the temporal and occipital lobes. There appears to be a lack of lateralisation to cortical changes in both younger children and recently diagnosed children, which supports the idea that RE is not a focal epilepsy, in terms of structural changes, which are focal and usually restricted to one lobe of the brain. It is quite likely that some, though not all of these regions, outside of the central sulcus are implicated in seizure generation. The findings in long term remission suggest that sparse regions of thicker cortex persist.

3.5.3 White matter studies

The white matter studies suggest that RE is not only a disorder of grey matter but also of the white matter layers which connect cortical areas. These changes are far-ranging and involve large white matter tracts in the perisylvian networks and the internal capsule and thalamic radiations. Smaller white matter structures such as the external capsule, fornix and midbrain appear to be unaffected. In voxel-based morphometry studies the white matter of the frontal, temporal and parietal lobes show regions of reduced FA. Younger children with RE were more likely to have lateralised differences in white matter microstructure (FA and MD) whereas older children with RE had more evidence of bilateral changes (both in voxel-based morphometry and TBSS). Limited differences were detected with TBSS analysis in groups with a duration of epilepsy over 3.3 years. Despite these insights, our understanding of how maturity and seizure remission affect white matter structures are hindered by the absence of longitudinal studies.

Normal white matter development starts in the second trimester of pregnancy and continues throughout childhood well into the third decade of life (Fields, 2008; Dubois *et al.*, 2014). Post-mortem studies suggest that white matter maturation initiates and develops in a central to peripheral progression. Maturation occurs first in proximal pathways compared to distal ones, second, in sensory pathways before the motor and third, in projection tracts before association tracts (Kinney *et al.*, 1988). Diffusion-weighted MRI can be used to measure the supposed integrity of the myelin sheaths of the oligodendrocyte and the axons they are wrapped around. This is because the coherent bundling and pruning and myelination of axons by oligodendrocytes alter the MR diffusion metrics (Feldman *et al.*, 2010). Mean diffusivity is believed to be a measure of membrane density and structure within axons. A decrease in MD is related to myelination, axonal packing and maturation. Fractional anisotropy (FA) is regarded as a measure of white matter integrity. It increases with myelination of axons and with an increase in axonal packing density. A large longitudinal DTI study of 159 healthy children (4-11 years) showed a posterior-to-anterior gradient of change. Furthermore, the greatest decreases in MD and increases in FA occurred in the frontal lobes, and the rate of change in FA is quickest within the left hemisphere (Krogsrud *et al.*, 2016). In comparison, this review suggests

that in children with RE, the developmental pattern of white matter in sensory and motor pathways and the peri-Sylvian networks of children with RE is aberrant.

In children with RE, there are areas within the white matter where FA was low and MD high compared to controls. Altered FA and MD occurred predominantly in the frontal and parietal regions, either unilaterally in voxel-based morphometry studies (Ciumas *et al.*, 2014) or bilaterally in tractography studies (Besseling, J. *et al.*, 2013). Furthermore, affected tracts (identified by TBSS analysis) predominantly included regions of the left superior longitudinal fasciculus (SLF), an association tract, and regions of the left and right internal capsule and thalamic radiations (IC), which are projection tracts. From these data, it would appear that the early posterior to anterior and central white matter development, which includes the optic radiation, midbrain, external capsule and fornix are normally occurring. Conversely, association and projection tracts, predominantly over the left hemisphere, appear to be aberrant with evidence of increased MD and decreased FA. These findings suggest a delay in the development of white matter which occurs after a period of normal early white matter development in the children with RE.

The differences in white matter may be attributed to aberrant development. The last step in the process of white matter development is myelination, which is a complex process that involves the interaction of oligodendrocytes and axons from the cortex. After a period of axonal overproduction, precursors of oligodendrocytes proliferate and migrate aligning with axons and forming initiator processes (Volpe, 2008). A single oligodendrocyte can myelinate multiple axons suggesting that each axonal process regulates its own myelination (Friede, 1972). It has been suggested that part of the control of this development is by neuronal maturation and their electrical activities. Indeed, in vitro mouse studies (Demerens *et al.*, 1996) have shown that blocking electrical activity inhibits myelination and an increase in neuronal activity enhances myelination. It could be possible that the changes detected by diffusion MRI in children with RE are a result of poor neuronal development and inter-ictal discharges within specific regions. There is also a possibility that these changes relate to hyper-local degeneration of the white matter, which can occur as a result to loss of oligodendrocytes, axonal loss or electrical changes (Barres and Raff, 1993; Semple *et al.*, 2013). These two possibilities are difficult questions to answer without investigating different groups of similar aged children with significantly

different duration of epilepsy in a longitudinal design. To understand the findings of this review, the TBSS studies were stratified with respect to age and epilepsy duration.

In younger children with RE, the white matter structures affected are predominantly lateralised in the left hemisphere and involve large white matter tracts, except the corpus callosum. However, in older children with RE, the findings were mixed; bilateral differences were more apparent in some studies, and in others the differences were rare or non-existent. These differences may be due to the time required for white matter abnormalities to develop (Feldman *et al.*, 2010) or may be due to the duration of epilepsy. The longer the duration of epilepsy, the fewer white matter differences are seen, visually as demonstrated in Figure 16.

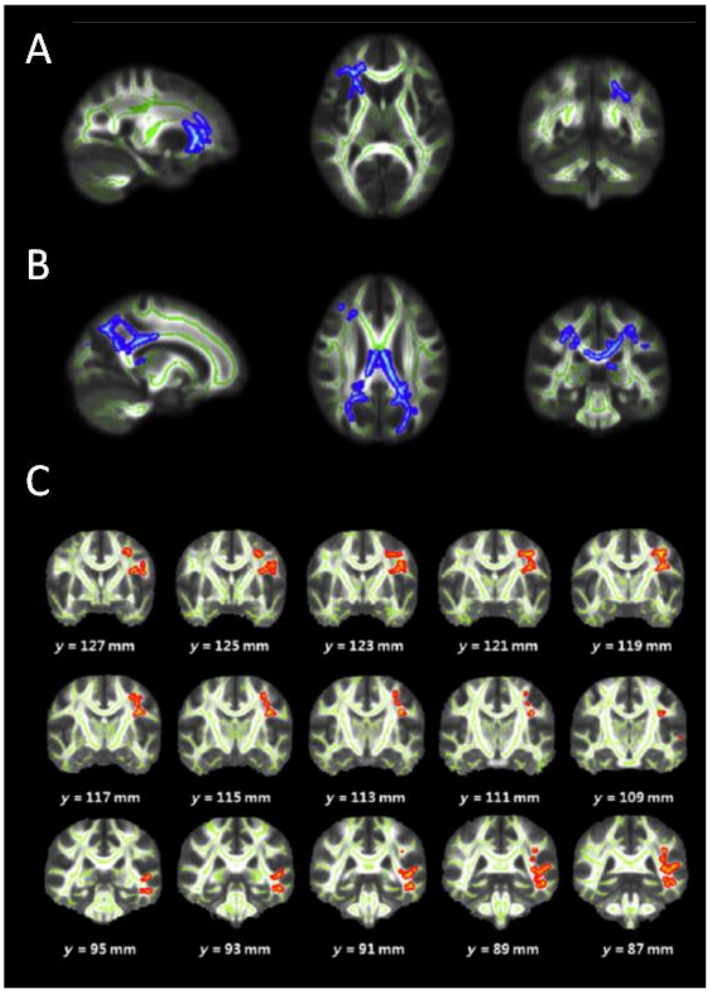


Figure 16: Diffusion-weighted MRI group differences in mean diffusivity detected by TBSS related to the duration of epilepsy (duration years). Xiao et al.: Sagittal, axial and coronal slices Group A (1.26±0.5), B. Group B (1.16±0.10). Increased MD (blue) was seen in the corpus callosum, bilateral cingulate gyrus and superior longitudinal fasciculus. Kim et al., coronal slices: C (3.3±1.5). Increase MD (red) is restricted and lateralised to the left longitudinal fasciculus, the rentrolenticular part of the internal capsule, posterior thalamic radiation and sagittal stratum. Y slice positions in mm.

These findings weaken the argument that seizures/electrical changes are damaging white matter and strengthens the argument that white matter tracts are maturing as the seizure disorder progresses. Moreover, it raises the possibility that the white matter differences in recent-onset epilepsy could be due to delayed development before the onset of epilepsy. These findings in affected white matter structures appear to have parallels with other neurodevelopmental disorders

Several neurodevelopmental disorders have similar white matter findings to those in children with RE. A decrease in FA and volume in the SLF has been reported in children with dyslexia (Vandermosten *et al.*, 2012), ASD (Catani *et al.*, 2016) and developmental coordination disorder (Langevin *et al.*, 2014). Interestingly, in typically developing children, an increase in FA in the SLF is associated with improvement in language and attentional abilities (Urger *et al.*, 2015) suggesting that maturity of this white matter tract is necessary for these cognitive improvements. An improvement in FA with brain maturation provides more evidence to view RE as a disorder of neurodevelopment, and aberrant development is leading to cognitive problems in these individuals. As there is some evidence to suggest that cognition in RE improves with seizure remission, it would be of interest to see if the SLF matures at the same time. To truly prove this requires a longitudinal study, with a baseline during the active epilepsy period and with a follow-up in seizure remission.

Overall, the findings from white matter studies in RE suggest that white matter changes are prevalent and predominantly affect large white matter tracts. There is a suggestion of delayed development or pre epilepsy “damage” with increased MD and decreased FA. The white matter then appears to normalise with longer duration of epilepsy. Unfortunately, any further understanding of white matter is hindered by the lack of tractographic and longitudinal studies.

3.5.4 **Comparison between grey and white matter studies**

Together, the information derived from grey and white matter studies suggests that discrete, regionalised, uni or bi-hemispheric brain changes are part of the disorder. Differences with healthy children are apparent within and outside of the central sulcus (rolandic fissure) and predominantly affect the frontal and parietal cortex and underlying white matter areas. This study has identified association white matter tracts, which connect between the frontal and parietal regions or ascending projection fibres to the frontal lobe and sensorimotor cortex and descending projection fibres to the striatum and thalamus. These differences, put together, suggest a network of dysfunction, and there are reasons to speculate that these differences are most likely due to delayed development. This predominantly because with increased duration of epilepsy (2-4 years), both white and grey matter differences become less apparent. Furthermore, in individuals with long-term seizure remission, the cortex mostly appears to normalise, it can only be assumed the same occurs in white matter, but there are no studies. Evidence in the literature suggests that RE has a complex genetic aetiology (Strug *et al.*, 2009; Panjwani *et al.*, 2016). In particular, variants of the transcription factor Pax 6, which is involved in successful brain development (Georgala *et al*., 2011) appears to be strongly associated with centro-temporal spikes one of the hallmark electrographic features of RE (Panjwani *et al.*, 2016).

An explanation for aberrant neurodevelopment in children with RE may be due to overexpression of Pax6, as suggested by genetics studies in human and mice. In foetal mouse studies, overexpression of Pax6 caused cell-autonomous defects of late cortical progenitor proliferation in the brain (Manuel *et al.*, 2007). In particular, overexpression of Pax6 can lead to abnormal patterning in the cortex. In normal cortical development, early patterning of the cortex is controlled by two concentration gradients of transcription factor proteins; Emx2 and Pax6 (Bishop, *et al.* 2002). The careful balance of these gradients along the neocortical proliferative zone defines the motor, somatosensory and visual regions. Mouse studies have shown that the result of overexpression of Pax6 makes larger proportions of neural progenitors differentiate into the motor and sensory neurons (Bishop, Rubenstein and O'Leary, 2002). This is followed in an increase in the area of the motor and sensory regions in detriment to the visual cortices.

There are also hints that a similar genetic mechanism may have a role to play in the development of white matter. In studies using a neurosphere model, overexpression of Pax 6 appears to be sufficient to direct almost all cells independent of their region of origin towards neurogenesis (Hack *et al.*, 2004). If overexpression of Pax6 has a similar effect in vivo, this will result in a deficit of oligodendrocytes and a reduction in the brain's ability to myelinate sufficiently. Furthermore, neuro-oligodendrocyte interactions have shown that myelinated axons have an increased diameter, which would increase the speed of action potentials (Barnett and Larkman, 2007) and in a smaller subsection of oligodendrocyte precursor cells, there is evidence that they have control over inhibition and excitation in neurons (McTigue and Tripathi, 2008).

It is possible that the incorrect positioning and reduction in the numbers of oligodendrocytes precursors during development could lead to differences in white matter brain structures using diffusion-weighted MRI. This reduction may cause problems in maintaining white matter tract structural stability when white matter volume increases with age and tracts concurrently increase in length. Myelination in humans is an extended process as growth and myelination need to match each other. For example, in the visual system, the P100 visual evoked potential arises at around 6-8 months (Taylor and McCulloch, 1992). The P100 will remain stable into adulthood despite yearly increases in the distance between the retina and calcarine fissure (Dubois *et al.*, 2014). If Pax6 is a factor in the development of RE, then the ability to generate enough oligodendrocytes to complete their myelination of axons may be impaired. Delayed myelination would especially be the case in periods where there is a rapid expansion in white matter volume as is the case in males.

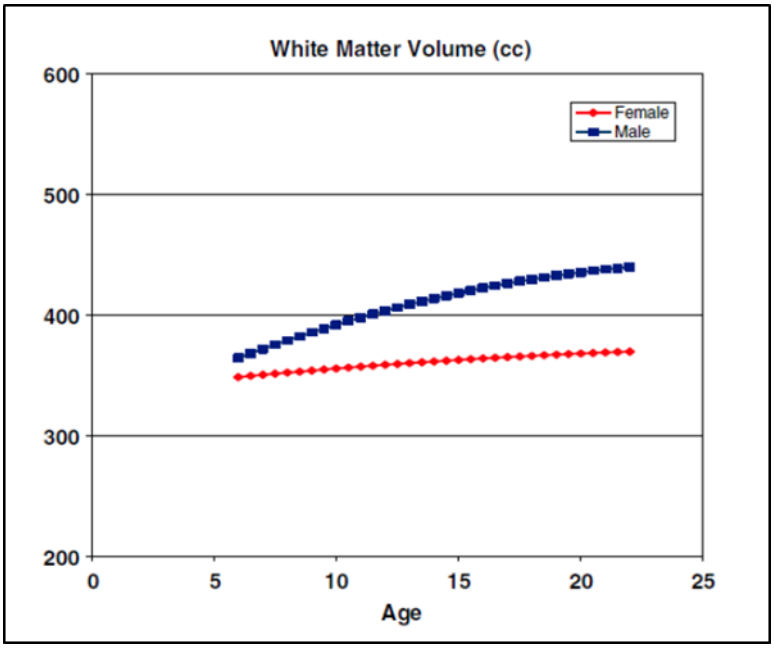


Figure 17: Longitudinal changes in global white matter volume with age. 224 females (375 scans) in red. 287 males (532 scans) in blue. The changes in males are larger and occur at a greater rate than those in females. Extract from (Lenroot and Giedd, 2006)

Extensive research suggests that males have a greater white matter volume than females, and the change in white matter volume is greater in males compared to females (Lenroot and Giedd, 2006). Although in both male and females there is an increase in white matter volume with age, this is at a greater rate in males, especially between 5-10 years. Interestingly most epidemiological studies indicate that males are disproportionately affected by RE compared to girls (Pal *et al.*, 2016) which makes the evidence of rapid increases in white matter volume a possible factor in the structural features seen in children with RE. It is possible that in individuals where there is a deficit of oligodendrocyte precursors, an excess of sensory and motor cortical neurons and rapid development of white matter volume then there is a risk of seizure development.

The combined findings of this review suggest altered neural development, which possibly starts before the onset of the epilepsy. It involves an extensive network of cortical and subcortical regions but predominantly involving the frontal and parietal regions and their white matter connections. Overall, this dysfunction of structure remediates with development even with increasing duration of epilepsy. It is postulated that the findings of this review would partly agree with the overexpression of Pax6 hypothesis which plays a part in influencing both cortical thickness of the frontal and parietal

lobes and the development of sufficient oligodendrocyte precursors. Excess PAX6 results in regions of excessive cortical thickening in younger children and a reduction in the ability to effectively myelinate tracts during excessive white matter growth. Reduced myelination would culminate with hyperexcitability of the motor and somatosensory cortex and dysfunction in the transmission of information along the affected white matter tracts.

3.5.5 **Study limitations**

Overall the methodological quality of the grey matter studies was limited. Five out of nine studies failed to report representative samples, and six studies failed to report the source of recruited controls. The mean age of the children ranged from 7 to 22.7 ± 2.7 years post-adolescent group C Pardoe *et al*. (2013) is very wide and includes those participants who are now in remission. The quality of methodologies was similarly in white matter studies. One out of the five studies used representative cases and sufficiently described the selection of their control group. Despite this, the use of covariates in their analysis process increased the overall quality. The age of the scanned children (9.4 ± 2.3 to 11.4 ± 2 years) was smaller in range and generally older than the grey matter studies but failed to include children at the ages of early or peak seizure onset (Panayiotopoulos, 2010). Quality of participant selection was average across both grey and white matter studies. In addition, there may be neuroimaging errors and methodological pitfalls that may have influenced these findings.

With regards to grey matter image analysis, the majority of the studies were interested in cortical volume, whereas only a few investigated cortical thickness. This limited sample made the interpretation of the cortical thickness results difficult. Furthermore, the quality of magnetic resonance imaging was poor. The slice thickness in six studies was over 1.2 mm, which can increase the signal to noise ratio, but also can also increase the likelihood of partial volume effect (McRobbie, Moore and Graves, 2007). An increase in partial volume effect can introduce error to the measurement of grey matter thickness and or volumes. In addition, the correct flip angle and time to repetition (TR) are crucial in reducing the signal to noise ratio (SNR). Two sites in the Pardoe *et al*. study used a long TR and short TE (1730 /4.38 ms) with a small flip angle (15°) which will produce a proton density image with little T1 weighting. Reducing the T1 weighting would introduce poor contrast between white and grey matter in these images and may have had a detrimental effect on the computational neuroimaging analysis. In white matter studies, neuroimaging techniques were better designed, but the analysis performed had limitations.

In white matter studies, there were few problems with the imaging methodologies. One unifying feature of the white matter studies was the use of tract-based spatial statistics (TBSS) (Smith *et al.*,

2006). This has been a very popular way of investigating white matter tracts at a group level. However, there are caveats with this technique, which may have influenced the results. In particular, the TBSS technique has problems with the visualisation of fine white matter structures as tracts crossing or voxels nearby to the tracts of interest can influence the resulting average FA skeleton (Bach *et al.*, 2014). Furthermore, the FA skeleton reduces complex tracts into simple one voxel sheet with no-directional information, this reduces the detection of significantly low FA values outside of the skeleton and increases the likelihood of non-white matter tracts influencing the FA skeleton (Bach *et al.*, 2014). Finally, the subsequent analysis of the FA skeleton commonly used the John Hopkins University (JHU) template (Oishi *et al.*, 2008) could lead to misinterpretation of the anatomy (Figure 18).

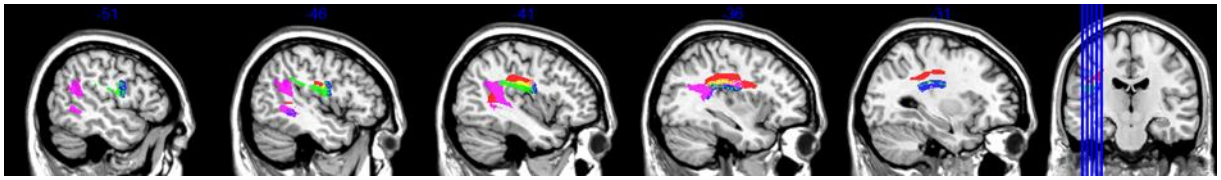


Figure 18: Visualisation of overlying white matter tracts from different anatomical atlases. Head and brain template from MRIcron with an overlay of tracts from two atlases. There is one tract from the JHU atlas (Oishi *et al.*, 2008) *superior longitudinal fasciculus (SLF) (red) and three from the Natbrainlab atlas* (Catani and Thiebaut De Schotten, 2008)*; arcuate long segment (blue), arcuate anterior segment (green) and arcuate posterior segment (pink). The crossover between tracts is coloured in yellow. There is limited cross-over between the JHU SLF and the Natbrainlab template furthermore; thus, demonstrating that the JHU atlas provides a very limited representation of the arcuate fasciculus.*

The main strength of this review is the comprehensive search using multiple layered search strategies and the stringent quality check using a modified Newcastle-Ottawa scale; however, there was difficulty in producing the correct inclusion criteria. One difficulty was on the reliance of controlled studies which removed several studies, some of which had found hippocampal asymmetries. Despite their removal, the selection of controlled studies strengthened the review. Age, gender, handedness and spike-location were all reported. Some of these variables could have been used as exclusion criteria, in particular, spike location or a cognitive deficit. It is the author's view that this would have unnecessarily complicated the review. Having said this, once a significant body of literature has formed these variables should be investigated in detail. To create a significant body of literature may involve a more detailed search for unpublished or foreign language literature.

In this review, a search for unpublished literature proved to be unsuccessful. However, this was expected because a large amount of time and money is expended in the production of these neuroimaging studies. As a result, it is suspected that there is a lack of publication bias in the literature, but this is difficult to prove. The inclusion of one non-English language paper (Japanese) is important because it raises the possibility that there are more non-English papers in existence. Overall, it is quite unlikely that there are more non-English papers as researchers want to disseminate their findings to the largest possible audience.

Ideally, a meta-analysis would have been performed on this data. However, due to the range of methodologies in both the grey and white matter literature, varying definitions of brain areas a lack of reproducible findings and the scarcity of raw data, a decision was made not to include this form of analysis. Hopefully, with time, an increase in the number of studies and the availability of detailed data with means and standard deviations will make meta-analysis a viable option. Nevertheless, this review attempted to clarify the findings of the neuroimaging literature in rolandic epilepsy both in a qualitative and where possible quantitative way. The knowledge generated by this review will hopefully lead the way in creating a hypothesis led approach to neuroimaging in this complex neurological disorder.

3.5.6 Implications of the review

Future research in this area needs to be improved both in quality and methodological considerations. Regarding quality, it is quite likely that the overall quality is higher than measured using the Newcastle-Ottawa scale because factors used to measure quality were not reported in the papers. Quality can also be improved by using representative cases. The range of neuropsychological problems associated with RE are well documented therefore, it does not make sense to exclude one of these cognitive deficits to produce a representative group. To produce a representative group in children with RE requires a reductionist approach; for example, RE and dyslexia only. Finally, sample quality was also affected by a lack of controlling for potential sources of variance. Sex, age and handedness information should be collected, presented in the paper, and at least age and gender should be included as covariates (Barnes *et al.*, 2010). In addition to the above, this review will propose recommendations to improve neuroimaging acquisition and analysis in the study of children with RE; these approaches have been utilised in this PhD study.

To improve T1 imaging studies, it is recommended that a 3T scanner is used. It should be able to acquire a slice thickness of 1.2 mm or less to improve the resolution of the image. A gradient-echo sequence with short time to echo (TE) and time to respond (TR) is the quickest way to produce a high-resolution brain image with good grey to white matter contrast which is essential for advanced neuroimaging analysis. Concerning the analysis of MR images, SPM (Ashburner and Friston, 2000) or FSL (Woolrich *et al.*, 2009) for cortical and subcortical volume and Freesurfer (Fischl, 2012) for cortical thickness are recommended, and the process of the analysis should be reported with as much detail as possible. The reporting of raw data such as cortical volume or thickness additional to whether an area has a significant difference compared to controls is recommended as this would allow meta-analyses to be performed in the future.

To improve the methodology of the diffusion-weighted imaging studies, a 3 T scanner which can produce equal to or greater than 30 gradients (with a B-value of 10,000 s/mm^2) is recommended. Even though TBSS is seen as state of the art for group analyses of white matter, it still has many flaws, and this may be hindering knowledge acquisition in non-lesional epilepsies. A sophisticated way to explore white matter tracts requires a tractographic technique such as diffusion tensor or

kurtosis imaging (Mori and Zhang, 2006; Wu and Cheung, 2010). Tractography is better at selecting specific tracts by using eigenvectors of diffusion data and the resulting directional eigenvalues. This approach to diffusion-weighted image analysis does, however, require apriori hypothesis, but this will make for better science. Also, an internal control white matter structure such as the optic radiation should be used particularly in a longitudinal study.

There are a few features that should be included in both grey and white matter studies. Currently, there is a crisis in neuroimaging with a lack of reproducibility in studies, which has been demonstrated in part with this review. To counter this, new strategies are required; for example, effect-size maps should be published so that findings no probabilistic significance, but a large effect size could be pursued in subsequent studies. In addition, multiple attempts should be made to replicate important findings rather than a casual acceptance of a disproved hypotheses. Furthermore, a consideration of spike side as a covariate may be of use, however, as there is evidence to suggest that these tend to migrate with time (Ewen *et al.*, 2011) it may matter little to the neuroimaging of individuals with RE. Nevertheless, to improve understanding of the effect of spike side as well as other changes over time, such as seizure remission the use of longitudinal study design will ensure the most rigorous and conclusive findings.

In conclusion, this review has identified a range of published controlled studies investigating grey and white matter structures in rolandic epilepsy. The data collected had limited quality, and there is room for improvement for future studies. Despite these failings, it appears that future targets for further research include the middle and inferior frontal gyri, putamen and caudate, the superior longitudinal fasciculus and internal capsule. Further exploration of these targets could prove useful in the understanding cognitive deficits and, potential seizure mechanisms in Rolandic epilepsy, which in time could be utilised as therapeutic targets.

4 Study cohort overview

This thesis will present data derived from measurements of brain structure, function and cognition in individuals with RE and healthy controls. The individuals with RE were recruited from NHS hospitals in England, whereas the controls were recruited by advertisement from within London. The additional participants with RE included in the cognition chapter were derived from the Genetics of Human Epilepsy study based at Kings College London. An attempt was made to acquire three different measurements from each of the RE participants which included, magnetic resonance structural neuroimaging, neuropsychology and EEG data. The controls had the same measures except for the EEG recordings. Each of these measures will represent a separate chapter.

Unfortunately, due to the complexity of the research, not every participant had the same measurements; indeed, there was difficulty in obtaining longitudinal measurements. This chapter will detail the cohort characteristics and which measurements were collected and how much overlap there were between the chapters. The first table incorporates the overlap between the different chapters for the participants with RE, the second table details the overlap for the control participants and the third details the individuals where only neuropsychological data was available at the baseline.

No socio-economic data were collected for this study, and as a result this is a limitation on the interpretation of the results. Nevertheless, it is the author's impression that many of the patients and the controls were from middle-income families. Furthermore, no *a priori* power calculations were performed at the beginning of the project, so it is likely that this study is underpowered.

Participant	Sex	Handedness	Age of first seizure	Seizure description	MRI-B	MRI-F	EEG-report	EEG-B	EEG-F	Npsych-B	Npsych-F
17-1058-301	M	R	9y,4m,29d	Loss of control of mouth, dribbling, eyes-rolling. Can generalise. Can occur day or night.	12.59	No	Yes	No	No	Yes	No
18-1057-301	M	R	9y,6m,0d	Feeling of his face freezing and stiffening of the neck. He starts to drool, followed by twitching of the lateral side of the right eye and side of the mouth. During the episode his tongue tastes like metal/stone. Last < 1 minute. Can't move and can't speak. Speech slurred for around 30 minutes afterwards. Occur in the daytime or before falling asleep. Can involve either left or right side of the face.	11.64 (artefactual)	No	Yes	11.61	No	Yes	No
19-1050-301	F	R	11y,2m,4d	Pins and needles of the tongue. The left side of the face goes into spasm or droops. Slurred speech. Around 1-2 minutes. Numb left arm and tingling in the fingers. Fully conscious throughout.	14.35	19.52	Yes	No	No	Yes	Yes

Participant	Sex	Handedness	Age of first seizure	Seizure description	MRI-B	MRI-F	EEG-report	EEG-B	EEG-F	NPysch-B	Npsych-F
20-1061-301	M	R	9y,9m,0d	Tongue tingles. Rt hand and arms shake. Makes gurgling and choking sounds. Fully conscious. Occurs in the daytime.	10.69	No	Yes	No	No	Yes	No
23-1075-301	F	R	7y,9m,13d	Feeling cold in her arms and legs. Shivering was unable to talk. Most of her seizures she is found lying on her side. Leg and arms are jerking. Eyes rolled back, pale, hypersalivation and rasping breath. All occur from sleep	11.64	13.3	Yes	No	No	Yes	No
24-1076-301	M	R	7y,7m,10d	GTCS from sleep	11.45	13.29	Yes	No	No	Yes	No

Participant	Sex	Handedness	Age of first seizure	Seizure description	MRI-B	MRI-F	EEG-report	EEG-B	EEG-F	NPysch-B	Npsych-F
26-1078-301	M	R	7y,2m,14d	No talking, slurred speech and no coordination. Drooling out of the side of the mouth.	13.91	No	Yes	No	No	Yes	Yes
27-1090-301	M	R	4y,6m,14d	Occurs from sleep. Lasts 2-3 minutes. Stiffening of fists and, clenching of teeth, foam, saliva and phlegm. Left-sided. Gurgling and choking. One episode lasted for 2.5 hours.	10.22	15.30	Yes	No	15.39	Yes	Yes
31-1091-301	F	R	8y,10m,3d	Awake: Face jerking right side. Arms try to push. No speech but was aware. Afterwards slurring with fatigue.	10.04	11.59	Yes	No	15.41	Yes	Yes
32-1099-301	M	R	8y,9m,24d	Minty sensation. His eyes roll upwards. His mouth deviates to the left. Remains conscious. Can have additional guttural sounds. Episodes of speech arrest with no other phenomena have been seen.	9.61	No	Yes	9.23	13.26	Yes	Yes

Participant	Sex	Handedness	Age of first seizure	Seizure description	MRI-B	MRI-F	EEG-report	EEG-B	EEG-F	NPysch-B	Npsych-F
33-1053-301	M	R	6y,3m,14d	Occurs from sleep. He wakes up and goes to the toilet. Upon returning to bed, the seizure will occur. Left-sided facial twitching, drooling with choking sounds can't talk. Sometimes aware of seizure. Can also have left-sided numbness in arm and leg. Has had an episode of status with 15 seizures in a row.	9.31	No	Yes	No	14.34	Yes	Yes
36-1100-301	F	R	7y,4m,13d	Occurs both from sleep and while awake. Throat constricted and left side of her face falls without twitching. Conscious throughout but unable to speak or move the muscles in her face. It lasts for around 30 seconds. Fine afterwards.	9.87 (artefactual)	No	Yes	No	13.70	Yes	No

Participant	Sex	Handedness	Age of first seizure	Seizure description	MRI-B	MRI-F	EEG-report	EEG-B	EEG-F	NPysch-B	Npsych-F
37-1101-301	M	R	10y,6m,6d	Occur from sleep. Whole-body jerking, mouth to left side jerking. Choking hypersalivation. Tries to speak but it is slurred. Lasts under 2 minutes. Goes back to sleep afterwards.	12.02	No	Yes	No	No	Yes	No
45-1042-301	F	R	7y,7m,27d	As a young child used to have episodes of vomiting from sleep. Occur from sleep. Tingling in tongue and cheek. Makes a clicking sound with her throat. Hypersalivation and her throat goes into a spasm. Left side mouth spasm. Aware but cannot speak. Lasts 1-2 minutes. Can affect left leg and arm.	9.80	14.22	Yes	No	14.21	Yes	Yes

Participant	Sex	Handedness	Age of first seizure	Seizure description	MRI-B	MRI-F	EEG-report	EEG-B	EEG-F	NPysch-B	Npsych-F
48-1039-301	M	R	7y,0m,0d	From sleep. Has tingling in tongue and cheek and is able to talk. Falls onto the floor in a foetal position, hand clenched. Eyes open and fixed. Makes gurgling noises and appears to be struggling to breathe. Lasts for 6-8 minutes. Afterwards, confused and scared can vomit. Daytime episodes of eye twitching, face feeling funny, eye-rolling, stumbling, staring. These are associated with tingling in the mouth, headaches, nausea, disorientation, poor coordination and disordered speech.	10.37	14.65	Yes	No	14.64	Yes	Yes
51-1103-301	M	R	4y,6m,18d	Occur from sleep. Looks grey and pallid. Tingling in tongue and cheek. Choking, gurgling, drooping of the left side of face. Aware but cannot speak. Breathing appears to stop and can become cyanosed. His eyes roll, and his body goes rigid and shakes. Last 3-4 minutes. Once recovered jerking can be apparent in the legs for up to 20 minutes. Rare post-ictal amnesia and	12.64	16.78	Yes	No	16.26	Yes	Yes

Participant	Sex	Handedness	Age of first seizure	Seizure description	MRI-B	MRI-F	EEG-report	EEG-B	EEG-F	NPysch-B	Npsych-F
53-1110-301	F	R	8y,9m,28d	Occurs from sleep. Wake up had a strange feeling in her stomach. Stiffening of her body and body shaking with LOC. On one occasion, only the left side convulsed. Afterwards poor word retrieval. Can stop breathing up to 2 minutes. Can be preceded by diarrhoea.	8.98	No	Yes	No	No	Yes	No
54-1015-301	M	R	7y,4m,9d	Occur from sleep or in the daytime. The left side of face droops and twitches with a wobbly jaw. Hypersalivation and aware but cannot speak, makes guttural noises. Sometimes has a numb left hand or arm. Episodes of a "fizzy" chin as he is falling asleep. During the day a "fizzy chin" combined with a sensation of enlarged tongue.	10.92	15.42	Yes	7.72	15.00	Yes	Yes
61-1112-301	M	R	2y,5m,14d	Occurs in the daytime only. Right-sided twitching of the mouth. Can walk around and understand what people are saying to him. Choking noises and hypersalivation. Seizure duration 15 minutes. Worse seizure 45 minutes ended up in hospital.	8.9 (artefactual)	13.01	Yes	8.39	11.91	Yes	Yes

Participant	Sex	Handedness	Age of first seizure	Seizure description	MRI-B	MRI-F	EEG-report	EEG-B	EEG-F	NPysch-B	NPsych-F
63-1118-301	M	R	7y,2m,14d	Occur from sleep. Tingling on both sides of the face. The right side of face droops. Dribbles and has jerking of the face. Feels like they can't breathe. After the event face still feels numb. This will last for 30 seconds. At times he can vomit.	8.11 (artefactual)	11.54	Yes	No	11.10	Yes	Yes
64-1114-301	M	R	6y,11m,18d	Arise from sleep. Wakes up and has a feeling that he will have a seizure. Has drooling from the mouth (unknown) side. Is aware but unable to speak. Seizures are usually under 10 minutes. On occasion had a 45 minute which required hospital admission.	9.34	No	Yes	No	No	Yes	No
65-1115-301	F	R	5y,6m,0d	Events occur early in the morning. No sensory, just motor phenomena. Right-sided twitching of the eyes and mouth with hypersalivation and gagging sound. Lasts a few minutes. She has awareness, but it is clouded (sleep like). Afterwards is sleepy. Can secondary generalised with clenched fists. Speech can be slurred for up to 20 minutes. Afterwards goes back to sleep.	7.95	11.44	Yes	6.10	No	Yes	Yes

Participant	Sex	Handedness	Age of first seizure	Seizure description	MRI-B	MRI-F	EEG-report	EEG-B	EEG-F	NPysch-B	NPsych-F
66-1116-301	M	L	8y,11m,14d	When younger had reflex anoxic seizures. Fainting when cold or in pain. His rolandic seizures arise from sleep. He gets a fizzy sensation around the tongue, lips and back of the mouth. The side changes between seizures. In addition, has episodes of going pale, blank staring, going rigid and falling to the floor. Eyes rolling and gently shaking. Worse event 10 minutes long. After a seizure he is confused and upset.	11.76	14.55	Yes	9.21	13.50	Yes	Yes

Table 27: Overview of participants with Rolandic epilepsy. Included in the table are the participant code, sex, handedness, age at first seizure and description of their rolandic seizure. Furthermore, included are whether the participants had MRI, EEG and neuropsychological (Npsych) data collected at baseline and follow-up and whether a copy of a clinical EEG report was available at baseline. Blue shading indicates "yes" and red "no". If the participant had an MRI or EEG, the age of individual when the data was collected is included.

Participant	Sex	Handedness	MRI-B	MRI-F	Npysch-B	Npsych-F
01-9206-301	F	R	12.19	No	Yes	No
02-9207-301	M	R	13.28	19.23	Yes	Yes
03-9202-301	F	R	14.41	20.53	Yes	Yes
04-9201-301	M	R	10.42	No	Yes	No
05-9216-301	M	R	13.78	19.09	Yes	Yes
06-9208-301	F	R	11.34	No (had a brace)	Yes	Yes
08-9209-301	M	R	11.56	16.99	Yes	Yes
09-9210-301	M	R	12.94	No	Yes	No
10-9203-301	M	R	13.16	No	Yes	No
11-9212-301	F	R	13.75	19.44	Yes	Yes
12-9204-301	M	R	13.32	18.51	Yes	Yes
14-9214-301	F	R	11.24	17.14	Yes	Yes
15-9215-301	F	R	12.89	No	Yes	No
16-9205-301	F	R	13.88	19.40	Yes	Yes
29-9218-301	F	R	13.08	17.51	Yes	Yes
46-9219-301	M	R	12.01	No	Yes	No
47-9220-301	F	R	13.71	17.65	Yes	Yes
56-9221-301	M	R	11.60	No (metal injury in the eye)	Yes	Yes
58-9222-301	M	R	10.31	14.42	Yes	Yes
59-9223-301	M	R	8.10	12.47	Yes	Yes
70-9224-301	F	R	8.68	No	Yes	No

Table 28: Overview of control participants. Included are the participant code, sex and handedness and whether the participant had an MRI and/or neuropsychological (Npsych) testing. Blue shading indicates "yes" and red "no". If an MRI scan was collected, then the age of the participant on the scan date was included.

Participant	Sex	DCDQ'07	TOWRE	CBRS	Age to first walk unaided	Age to first speak a two-word sentence
1001-301	Male	Yes	No	No	Yes	Yes
1003-301	Male	Yes	No	Yes	Yes	Yes
1006-301	Female	Yes	No	No	Yes	Yes
1010-301	Male	Yes	No	Yes	Yes	Yes
1011-301	Male	Yes	No	Yes	Yes	Yes
1020-301	Male	Yes	No	No	Yes	Yes
1023-301	Female	Yes	No	No	Yes	Yes
1025-301	Male	Yes	No	Yes	Yes	Yes
1032-301	Female	Yes	No	Yes	Yes	No
1035-301	Male	Yes	No	Yes	Yes	No
1041-301	Male	Yes	No	No	Yes	Yes
1047-301	Male	Yes	No	Yes	Yes	Yes
1068-301	Female	Yes	No	Yes	Yes	No
1069-301	Female	Yes	Yes	Yes	Yes	Yes
1072-301	Male	Yes	Yes	Yes	Yes	Yes
1082-301	Female	Yes	No	No	Yes	No
1087-301	Female	Yes	Yes	No	Yes	Yes

Table 29: Characteristics and measurements of additional participants with Rolandic epilepsy. Included are the participant code, sex, handedness and whether they completed the developmental coordination disorder questionnaire '07, Test of Word Reading Ability (TOWRE), Connor's Behavioural Rating Scales (CBRS) and answered questions on

the age to first walk unaided and first speak a two-word sentence. Please note that the handedness data for these participants are unknown. These participants were analysed in Chapter 5.

5 Developmental coordination disorder and other specific learning disabilities in children with Rolandic epilepsy in active epilepsy and seizure remission

5.1 Introduction

5.1.1 Rolandic epilepsy and developmental coordination disorder

Rolandic epilepsy (RE), is a presumed neuro-developmental disorder which is associated with disordered movement and DCD. As presented in Chapter 2 the prevalence of DCD is high (Kirby *et al.*, 2017) and it appears that the children may have motor dysfunction before their first seizure (Scabar *et al.*, 2006; G. M. Overvliet *et al.*, 2011). There may be an association between the density of the Rolandic spike and the severity of the DCD (Vannest *et al.*, 2016). Furthermore, the location of spikes on the EEG does not associate with whether a child will have a motor deficit (Ayaz *et al.*, 2013). What is unknown and what this chapter will explore is whether there is evidence to support the persistence of developmental coordination disorder into seizure remission.

Currently, the evidence for DCD in seizure remission is limited. There is evidence for a group improvement in fine motor function, but they do not reach control performance (Garcia-Ramos *et al.*, 2015), and some individuals may have dysorthographia (Massa *et al.*, 2001). The Garcia-Ramos study had 71.4% in seizure remission, and the Massa study found dysorthographia in a group of children with RE and disordered cognition. Therefore, there is a gap in the literature; there is a need to identify the prevalence of DCD in this population, to see if there is any evidence of motor disorder before developing seizures, to identify what co-occurs with DCD and whether these problems persist into seizure remission. The following section will detail developmental coordination disorder and its association with seizures and epilepsy.

5.1.2 Developmental coordination disorder

Developmental coordination disorder (DCD) is a neuro-developmental disorder characterised by difficulties with the acquisition and performance of age-appropriate gross and fine motor skills, in the absence of any known medical condition or neurologic dysfunction (American Psychiatric Association, 2013). It is one of the most common disorders affecting school-age children with a prevalence of 5-6% (American Psychiatric Association, 2013) which can manifest with delays in developmental motor milestones such as standing and walking, sub-standard performance in sporting activities and untidy handwriting (Kirby and Sugden, 2007). The disorder appears to have sex bias with males more likely to be affected than females with reported ratios of 1.9:1 (Lingam *et al.*, 2010). Furthermore, there is a greater prevalence of DCD in risk families with a lower socio-economic background, individuals with low birth weight (<2500g) or parturition before 37 weeks gestational age (Lingam *et al.*, 2010) and a delay to independent walking greater than 15 months or later (uncorrected for gestational age) (Faebo Larsen *et al.*, 2013). DCD appears to indicate dysfunction of brain motor systems. Interestingly this disorder seems to frequently occur with other deficits of cognition, which may indicate a generalised dysfunction of the brain or shared cognitive mechanisms.

Developmental coordination disorder appears to frequently co-occur with one or more disorders of neurodevelopment and cognition. The most common of these include dyslexia, specific language impairment (SLI), otherwise known as developmental language disorders (DLD), attention deficit hyperactivity disorder (ADHD) and autism. The relationship with DLD is strong with one review stating that there is “substantial co-morbidity between DLD and poor motor skills and that the motor deficits seen are similar to those in DCD (Hill, 2001). There is good evidence to suggest that these disorders frequently overlap and have similar deficits (Hill, 2001; Bishop, 2002; Archibald and Alloway, 2008). There is an apparent relationship with dyslexia; after controlling for potential confounding factors, a study found children with DCD had an association with reading (OR:3.35[2.36-4.77] and spelling (OR:2.81[2.03-3.90] problems (Lingam *et al.*, 2010). The overlap between ADHD and DCD has been estimated to be as much as 50% (Gillberg *et al.*, 2004). DCD

appears to be frequently comorbid with other disorders of cognition, interestingly individuals with this disorder can have one or more of these comorbidities.

Multiple comorbidities appear to be the rule, not the exception, and this could suggest that motor problems and DCD are the results of a more generalised disorder of neurodevelopment. Kaplan found in a group of 115 children referred because of learning and attention problems but not motor problems, 81 had DCD, and within the DCD group, 28.3% had both ADHD and dyslexia (Kaplan *et al.*, 1998) (Figure 19). These findings could indicate that DCD is the most prominent neurodevelopmental disorder, but this may be because of its visibility. There is evidence to suggest that DCD and its co-occurring cognitive issues may persist into adulthood.

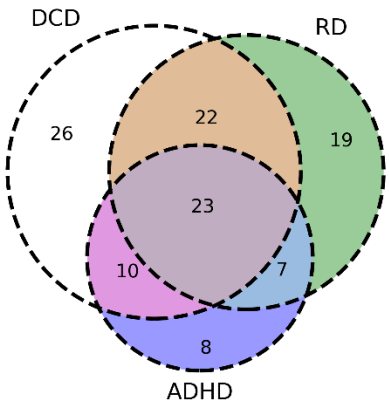


Figure 19: Venn diagram of comorbidities in children referred for learning and attention problems. This figure demonstrates that DCD is the main issue in these children with ADHD and reading disability (RD) frequently co-occurring. Data extracted from Kaplan *et al* (1998).

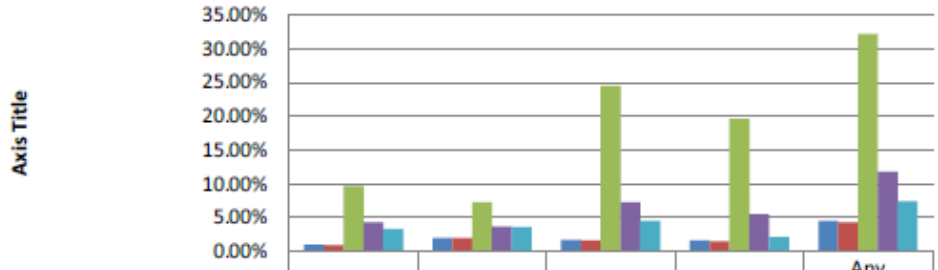
There is increasing evidence that children with DCD have persisting motor difficulties as they become adolescents and adults, and this may include their comorbidities. In a longitudinal controlled study by Cantell, adolescents with DCD had lower Weschler Adult Intelligence scores and shorter school careers (Cantell, Smyth and Ahonen, 2003). Similarly, in a study of a group of adults with DCD, they performed more poorly than controls across all motor tasks, in particular, these individuals had problems with speed, the variability of their movements, sequencing and dual-task performance (Cousins and Smyth, 2003). Problems with speed were particularly the

case in the performance of a new unlearnt task (Cousins and Smyth, 2003). Despite the questionnaire and qualitative data suggesting other cognitive problems, there is little longitudinal quantitative neuropsychological evidence exploring comorbidities in DCD and whether they improve with age. Similarly, there are few cross-sectional studies which investigate motor function in adolescents and adults with dyslexia, DLD and ADHD.

There is mixed evidence from cross-sectional studies of motor deficits in adults with either ADHD, dyslexia or DLD. In a comparison of motor movements between adults with dyslexia (n=19) and healthy age-matched controls (n=20), there is evidence that adults with dyslexia are slightly slower. Data collected using a Perdue pegboard found a small significant difference ($p<.05$) in speed but only in the dominant hand of adults with dyslexia (Thomson *et al.*, 2006). Whereas in ADHD, there is evidence of lateralised motor deficits which may persist with age. A PhD study which utilised the grooved pegboard to assess motor abilities in groups of children (n=17), adolescents (n=18) and adults (n=19) with ADHD found significant differences only in the non-dominant hands in the child ($p=0.002$) and adult ($p=0.006$) groups when compared to normative data (McHale, 2010). A review of the literature on DLD and SLI found an absence of evidence investigating motor function in adolescents and adults. Overall, adolescents and adults have limited evidence of disordered motor function; any evidence suggests a difference in deficits between dyslexia and ADHD.

Developmental coordination disorder is a neurodevelopmental disorder of unknown aetiology, which appears to have a strong association with other neurodevelopmental disorders such as dyslexia, developmental language disorders and attention deficit hyperactivity disorder, with a large proportion of individuals with DCD having one or more of these comorbidities. With development into adulthood, deficits in motor function can still be apparent; however, there is little quantitative evidence on what happens to other comorbidities with age. Similarly, there is a large gap in the literature relating to motor function in adults with dyslexia, DLD and ADHD. Overall, the evidence is strong for disordered movement to be inherent to a disorder of cortical function which may be the result of aberrant neural development and this may also be apparent in individuals who experience single seizure event or develop epilepsy.

5.1.3 Seizures, idiopathic epilepsy and developmental coordination disorder



	ASD	ADHD	DCD	LD	Any ESSENCE
Population	1.00%	2.00%	1.70%	1.60%	4.50%
Population without seizures	0.90%	2.00%	1.60%	1.50%	4.30%
Epilepsy	9.70%	7.30%	24.50%	19.60%	32.20%
Febrile seizures	4.30%	3.70%	7.30%	5.50%	11.80%
Febrile seizures only	3.30%	3.60%	4.50%	2.10%	7.40%

Figure 20: Prevalence of ASD, ADHD, DCD, Learning Disability (LD) and Early Symptomatic syndromes eliciting neuro-developmental clinical examinations (ESSENCE). Percentage prevalence for indication of DCD in middle of the figure. Total population (popn) prevalence (blue), prevalence in non-seizure popn (red), prevalence in popn with epilepsy (green), prevalence in those with febrile seizures and those who had febrile seizures only. Extract from Gilberg et al (2017). This chart demonstrates an increase prevalence for DCD in children with epilepsy, febrile seizure or febrile seizures only.

There is strong evidence that children with DCD are more likely to have seizures than other children. In a large Swedish study of 27,092 twins, 1.82% had febrile seizures, and 1.04% had epilepsy with 1.7% indicating DCD (Figure 20). Within the DCD population, 7.3% had experienced febrile seizures, and 24.5% had epilepsy, only 1.6% had DCD without seizures (Gillberg *et al.*, 2017). The study suggested that increased risk for DCD in children with febrile seizures was associated with the development of epilepsy (Odds ratio 3.8). Furthermore, if the child had epilepsy, increased risk for DCD was related with early diagnosis (OR: 2.8) and being male (OR: 2.8). Overall, DCD appears to have a strong association with epilepsy, what is unclear is which idiopathic epilepsies are most likely to be associated with DCD.

Epilepsy and specifically idiopathic epilepsies appear to be associated with disordered movement in both adults and children. In a study of adults with newly diagnosed partial (n=26, 65% unknown aetiology) or generalised epilepsies (n=26, 77% unknown aetiology) versus a control group (n=26) motor problems were apparent. Compared to controls, difficulties with the Perdue pegboard were seen in both the dominant (p=.01) and non-dominant (p=.02) hands (Pulliainen,

Kuikka and Jokelainen, 2000). A similar deficit can be seen in groups of children with idiopathic epilepsies. In a study by Herman *et al.,* they investigated motor function in children with newly diagnosed epilepsy (n=53) compared to healthy controls (n=50). They found motor deficits in children with epilepsy with the grooved pegboard task in both the dominant (p<.01) and non-dominant hands (p=<.05)(Hermann *et al.*, 2006). It is unclear what proportion of the cohort had which type of idiopathic epilepsy; despite this omission, motor problems appear to be the most prevalent cognitive issue. Both Herman *et al.* and Pullianien *et al.,* demonstrate deficits in motor function in individuals with epilepsy, however, it is unclear whether these deficits are apparent before the generation of seizures or occur as a result.

There is a deficit of studies investigating motor deficits before the onset of a seizure disorder. Hence, the following paragraph explores developmental delay as a potential indicator of DCD. In a Swedish study of children with a single seizure event (n=750), 21% of the children had evidence of developmental delay before or at their first seizure (Jason *et al.*, 2018). Furthermore, at follow-up two years later, the risk of a further seizure was 74%; despite this, the population prevalence of developmental delay was similar to baseline. If the child had evidence of developmental delay, this increased the risk of further seizures (OR: 2.42). This data suggests that development delay one of the indicators of DCD may be apparent before the generation of epilepsy, moreover, as there is no change to the prevalence of these issues on follow-up, it could suggest that seizures do not produce motor problems in those not already affected.

The following section will present the rationale for the chapter.

5.1.4 **Rationale**

Rolandic epilepsy is an idiopathic focal epilepsy which is associated with motor problems and developmental coordination disorder. Furthermore, this disorder frequently co-occurs with other specific learning disabilities (SPLDs) such as dyslexia, ADHD, DLD and central auditory processing disorders (CAPDs). It is unclear whether evidence for DCD precedes RE or follows the development of epilepsy and how it co-associated with other SPLDs.

This chapter aims to identify the prevalence of DCD in RE during active epilepsy and how this relates to delayed development. The prevalence of DCD and other SPLDs will be calculated and compared with control data. In addition, group neuropsychological test scores will be compared between the RE and control group. The RE group will be divided into those with a DCD and those without DCD and statistically compared. At follow-up, cross-sectional data collected from tests to investigate dyslexia and motor function will be collected. Furthermore, baseline tests will be repeated, and longitudinal analysis of the data sets performed. This chapter will identify specific cognitive problems in children with RE and measure how they change between active epilepsy and seizure remission.

5.1.5 **Hypotheses**

- Developmental coordination disorder will be more prevalent in children with RE compared to healthy controls.

- Delayed motor and speech development will be associated with developmental coordination disorder in children with RE

- The DCD will be more severe compared to the control population.

- Co-occurring cognitive problems will be more prevalent in children with RE group compared to the control group.

- The amount of overlap in the RE group between the co-occurring cognitive problems will be far greater than in the control group.

- In seizure remission, there will be a remission of the features of developmental coordination disorder.

5.2 Methods

5.2.1 Participants

The initial recruitment was of forty children with RE from tertiary epilepsy centres in England, the United Kingdom (UK). In addition, a further four children were recruited from the United States of America (US). The recruitment occurred between the periods of July 2005 and December 2014. They all had a diagnosis of RE based on the ILAE criteria (Engel, 2001). All of these children had more than one focal sensory and motor seizure with or without secondary generalisation and had evidence of rolandic spikes on their scalp EEG either while awake or in a sleep state. To be included in the study, the children had to have no other neurological problems and a normal brain MRI. Finally, every child had to have an IQ above 80 and had to pass a pure tone threshold hearing examination.

5.2.2 Neuropsychology measures

Several tests were used to identify and classify cognitive deficits in the participants. The choice of tests used in this study was defined by the work done by Dr Colm McGinnity and Dr Anna Smith during the baseline. This included the full-scale WASI IQ testing, Test of Word Reading Efficiency 2nd Edition, Developmental Coordination Disorder Questionnaire '07, SCAN-C and the Connor Behavioural Ratings Scale. In addition, data was collected for the Word Range Achievement Test 4th Edition (WRAT), Clinical Evaluation of Language Fundamental 4th Edition (CELF) but this was not in sufficient amounts to warrant baseline group analysis and longitudinal assessment. Most of the baseline data were collected in an interview room at the Kings College Hospital clinical research facility (KCH-CRF) or in the child's home. At follow-up, additional tests were performed which are detailed in this chapter. The data was collected in either an interview room at the KCH-CRF or an interview room at the Centre for Neuroimaging Studies (CNS) at Kings College London

prior to the follow-up MRI scan. The data was collected by myself and Dr Anna Smith. The data derived from the neuropsychological testing was used to define deficits.

The neuropsychological tests were used to define deficits in the participant's cognition if the deficit is large, then this can be defined as a specific learning disability (SPLD). The deficits were classified as either a moderate or severe SPLD. The cut-offs for moderate and severe SPLD were based on the methods used by the British Joint Council of Qualifications (BJCQ) in their identification of individuals who would require support in examinations due to impaired function (Joint Council for Qualifications, 2015). Using this measure a child with a standardised score of 84 or less (<1SD) would be classified as having an impairment and would be allowed 25% extra time in an exam. A child with a standard score of 69 or less (<2SD) would be classified as having a very substantial impairment and would receive 50% extra exam time. This classification system was created by the BJCQ so awarding bodies would comply with the duty under the Equality Act 2010 to make “reasonable adjustments” in examinations (Office for Disability Issues, 2013; Joint Council for Qualifications, 2015). Using the BJCQ system for the cut-offs in this study means that the findings will have real-world implications regarding educational and examination support. A series of neuropsychological tests and questionnaires were used to assess for SPLDs.

The developmental coordination disorder questionnaire 2007 (DCDQ’07) was used to test for the indication for DCD. Parents complete this questionnaire, which asks questions related to three key areas, control during movement, fine motor/handwriting and general coordination. This questionnaire can be used to provide an indication for DCD, but it is not a diagnostic assessment and only fulfils two of the diagnostic criteria for DCD (Barnett, 2008). Despite this, the questionnaire measure correlates well with other tests of DCD, which include the Movement ABC (r=0.55) and Test of Visual-Motor Integration (r=0.42). Furthermore, the DCDQ’07 has good sensitivity (85%) and specificity (71%) (Wilson *et al.*, 2009). Unfortunately, there is no way to classify the indication for DCD into moderate and severe impairment categories. The following tests allowed for classification.

The Test of Word Reading Efficiency (TOWRE) is used to identify problems with reading, which could be evidence of developmental dyslexia; in this study, the term reading disability (RD) was used. This assessment measures the participant’s ability to read words (sight word efficiency,

SWE) and non-words (phonemic decoding efficiency, PDE) under timed conditions. The SWE component measures lexical reading ability. Lexical reading involves an automatic identification and retrieval of known words and subsequent verbal production (Paap and Noel, 1991). The non-lexical pathways are more complex; the participants require the ability to identify graphemes and then convert them into phonemes (Paap and Noel, 1991). A review of the TOWRE found it to be a valuable instrument for the measurement of word reading efficiency for individuals aged 6 to 24 with good reliability in both SWE (0.91) and PDE (0.92) (Tarar, Meisinger and Dickens, 2015). To be classified with a moderate impairment for dyslexia, the participant needed to perform 1 SD (<85) below a normal standard score in either SWE or PDE. Classification of severe impairment was two standard deviations (<70) below the mean.

The Connors 3rd Edition Comprehensive Behaviour Rating Scales Parent Assessment report was used to identify if the participants fulfilled the diagnostic statistical manual 4th Edition criteria for either inattentiveness or hyperactive and impulsive behaviours seen in individuals with ADHD (American Psychiatric Association, 2013). This questionnaire is a thorough and focussed assessment of attention deficit hyperactivity disorder, co-occurring disorders and associated features. This questionnaire can be used to fulfil some of the criteria for a formal diagnosis of ADHD, a review of the parent report form found a correct classification rate of 84% (Kollins *et al.*, 2011). Inattentive or hyperactive and impulsive behaviours of greater than 1 SD of a normal T-score (>60) were classified as a moderate impairment. A T-score greater than 70 (2 SD) was classified as a severe impairment.

To test for central auditory processing disorders (CAPD), the SCAN-C for children and SCAN-3 test for adolescents and adults. This test assessed the ability to identify filtered words, words said with background speech babble and two dichotic listening tasks involving competing words and sentences. These tests are used to indicate CAPD and are not diagnostic. There are four categories in this test, therefore, to reduce the chance of false positives to be classified as having moderate CAPD the participants had to perform 1 SD below a normal standard score (<85) in two or more of tasks. If individuals scored below two standard deviations (<70) in two or more tasks, this was classified as severe CAPD. All of the participants had pure tone threshold testing of both ears performed before the SCAN testing both at baseline and at follow-up. To be included in the

results required the participant to have a normal range between 500 Hz and 4000 Hz at 25 decibels (dB).

All of the above measures were recorded during active epilepsy and repeated in the follow-up. In addition, extra testing was performed at follow up. Cut-offs were not used for these tests as the measures were interested in exploring individual and group differences. The additional follow-up tests were chosen by the author of this thesis. The following tests were included in the follow-up battery; the Comprehensive Test of Phonological Processing (CTOPP), grooved pegboard and the Detailed Assessment of Speed of Handwriting (DASH).

The CTOPP measured the ability to process and manipulate phonological information. In a review, it has been described as a reliable and valid measure of phonological processing for individuals aged 4-24 and is likely to yield clinically and educationally relevant information (Dickens, Meisinger and Tarar, 2014). The test is ideal for collecting evidence for dyslexia as it examines phonological awareness, phonological memory and rapid naming (Eden *et al.*, 2016). As dyslexia is frequently co-morbid with motor problems, two motor tasks were also included.

The grooved pegboard was used to measure fine motor function in the dominant and non-dominant hands. The grooved pegboard test has good test-retest reliability (0.91 and 0.85 for right and left hands, respectively) and correlates with the Bruininks-Oseretsky test (-.50) (Wang *et al.*, 2011). This test is more complex than other pegboard tasks as it requires manipulation of the peg to fit the hole, like a key in a lock. To assess dominant hand function and written communication skills, the detailed assessment of handwriting (DASH) was used. This test was used to see if written communication was poor, which may impede the ability of the child to succeed in education while in seizure remission. The DASH is a reliable measure of hand-writing speed, which has been used by occupational therapists to provide information on the nature and severity of the handwriting difficulties (Francis, Wallen and Bundy, 2016). To further understand the nature of the motor problems in RE, the parents were questioned about developmental delay.

Parents of the children with RE were asked questions about their child's early motor development. The questions identified the age of key developmental milestones; when their child first spoke a sentence of two words, and when they first walked unaided. These routine questions are part of a child development screen devised by the Royal College of Paediatric Health to assess whether

children are developing normally (Cole and Ball, 2013). The questions were used to understand whether the deficits in motor function were preceded by delayed development of speech and gross motor function. All the results from the RE group were compared with large normative data sets.

5.2.3 Statistics and data visualisation

Using SPSS Version 25 (IBM Corp) odds ratios and χ^2 significance tests were calculated for each SPLD in RE compared to healthy controls at both baseline and follow-up. The χ^2 test was used to test the difference in proportions between groups of indication for each SPLD. In the follow-up data, the Chi-squared test was used to identify if there were any within-group changes in the proportions of children with evidence for SPLD. Included with this analysis are graphical presentations of speech delay and motor delay and DCDQ scores. Using the python toolbox Venn3 within Jupyter notebooks (Thomas Kluyver, *et al*., 2016) a Venn diagram was created for both the Rolandic group and healthy control group. Venn diagrams were used to visualise the overlap of DCD, dyslexia and ADHD within the two groups.

A sophisticated analysis of the longitudinal data was included. A linear mixed-effects (LME) model otherwise known as a multi-level linear model, was used to measure change between baseline and follow-up neuropsychology scores for WASI matrices, TOWRE-2, SCAN, DCDQ'07 scores and Connors hyperactive and inattentive subtypes of ADHD. The LME model used the programming language R (R Core Team, 2012) and the script lme4 (Bates, Maechler & Bolker, 2012). A LME model was used as it avoids violating the assumption of statistical independence of observations, which is ideal for longitudinal measurements, and it allows for missing data from timepoints to be included in the model (Wainwright, Leatherdale and Dubin, 2007). Within the model, sex was used as a fixed effect (without an interaction term). Random effects included intercepts for participant number and time point". Slopes were also random. The output from the linear mixed effect model analysis is a Chi-Square value with an associated p-value; this is because the LME model involves the comparison of the full model with the effect of having a diagnosis of RE against a model without the effect (Figure 21).

```
PDE.null = lmer(TOWRE_PDE ~ Sex +
(1|Number) + (1|Time_point), data=lon_cog,
REML=FALSE)

PDE.model = lmer(TOWRE_PDE ~ Type +
Sex +
(1|Number) + (1|Time_point), data=lon_cog,
REML=FALSE)

anova(PDE.null,PDE.model)
```

Figure 21: Example of r code for running the linear mixed-effects model analysis. PDE.model (highlighted yellow) is the model with the effect of having Rolandic epilepsy (RE), PDE.null (highlighted blue) is the model without the effect of having RE. Sex is a covariate in both of the models. (1|subject) represents an assumption that there is a different intercept for each individual. (1|Time_point) represents an assumption that there is a different intercept at each time point. Sex was a fixed effect. REML=FALSE is a function required when you compare models using a likelihood ratio test. The green highlighted code is required to compare the two models.

5.3 Results

5.3.1 Baseline cohort

5.3.1.1 *Demographics*

Forty-four children were identified, but four did not have motor milestones, so they were removed from the sample. A further four were removed as they appeared to be in seizure remission (> 1year seizure-free) at the time of testing. Therefore, thirty-six children with Rolandic epilepsy and 22 healthy controls were recruited. All the children with RE had clear evidence of more than one focal seizure with or without secondary generalisation with evidence of rolandic spikes on scalp EEG. There was no evidence of other neurological problems.

	Controls (n=22)	RE (n=36)	Significant (p=)
Mean age (years ±SD)	12.09±1.68	10.57±1.68	0.0004
Sex (% male)	50%	63.8%	0.297
Righthanded (%)	22 (100)		
First seizure (years)	N/A	7.37±2.05	N/A
AED (%)	N/A	50%	N/A
WASI IQ	115.91±11.01 (n=22)	108.39±14.2 (n=31)	0.021
WASI Matrices	116.34±8.11 (n=22)	109.11±14.09 (n=19)	0.059

Table 30: Demographics of participants with RE and controls. Included are mean age, sex, handedness, age at first seizure, anti-epileptic drug use (AED) and Weschler Adult Intelligence Quotient (WASI IQ) scores. To avoid bias in the analysis of the data, individuals with missing data were removed in some of the analyses resulting in smaller sample sizes. Using a t-test here was a significant difference between the group in age and full-scale WASI IQ scores. Degrees of freedom: age and sex =57, WASI IQ = 52, WASI matrices = 40.

Demographic data and WASI IQ scores for both groups are contained in Table 30. There was a significant difference in age between the two groups. Despite this difference, the effect of age would be mitigated by the conversion of scores into standard scores, which were corrected for the participant's age. Furthermore, despite higher than average intelligence, there was a significant difference between the groups at baseline.

5.3.1.2 *DCDQ'07 scores and the indication for DCD in RE.*

Fifteen out of 35 children with RE (41.7%) had an indication for DCD at baseline compared to 3(13.6%) in the control group, odds ratio (4.75, CI 1.18 to 19.06, p=0.028). A comparison of DCDQ'07 scores between the RE group (58.23±15.58) and the controls (69.59±7.33) was found to be highly significant (p=0.001, d=0.93). The RE group DCD scores were compared with the age at first seizure (r=0.180, p=0.3) and duration of epilepsy (r=-0.25, p=0.156) but were found not to be significant.

5.3.1.3 *Developmental milestones*

The age of time to walk unaided and age to speak two-word sentence was compared with normative data from the literature. The mean age to walk without help in the children with RE was 11.83 ±2.1 months (n=36) and ranged between 8-17 months. A comparison with normative motor data recorded by the United Nations World Health Organisation (UNWHO) found in 816 healthy children the time to walking alone was 12.1±1.8 months; there was no significant difference (p=0.38) (De Onis, 2006). A lack of significance was found for the two-word sentence developmental milestone.

In the RE group (n=30)., the age to speak two-word sentences was 19.4±5.8 months with a range between 10-38 months. When compared to data from 59 typically developing children in Indiana, USA (Rudolph and Leonard, 2016) there was no significant difference (p=0.94). In the normative group, the mean time to speak a two-word sentence was 19.3±5 months; this was not significant with the RE

group. The data from this study for age at the time to walk unaided and age to speak a two-word sentence are presented in Figure 22 and Figure 23.

Figure 22 demonstrates a normal distribution for the onset of the behaviour. There was one participant with RE who was outside the 2.5 SD norm cut-off, which equated to 2.7% of the cohort. Figure 23, demonstrates a Poisson distribution of the data; there were two participants who exceeded the 2.5 SD cut-off, which equated to 6.6% of the cohort.

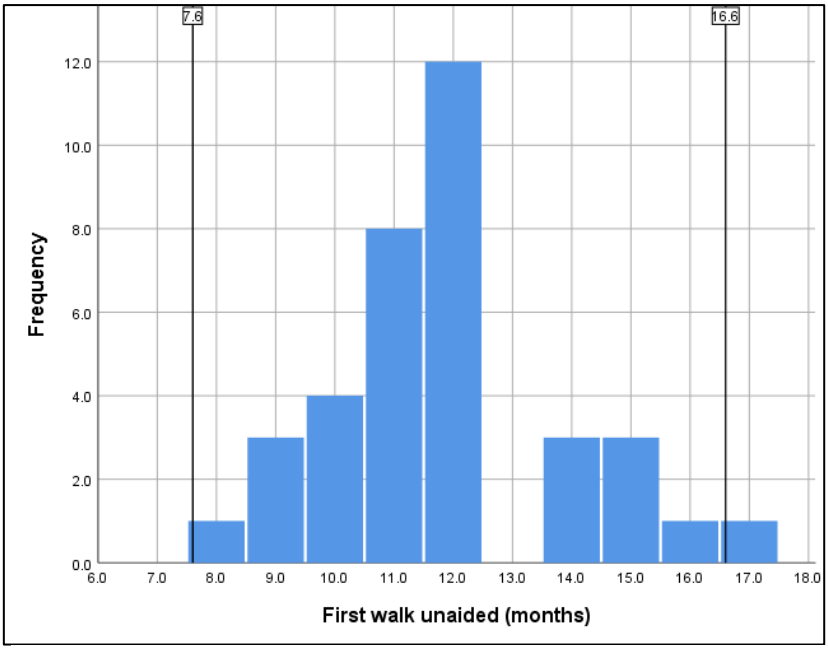


Figure 22: Age of children with RE first walked unaided.Vertical lines are 2.5 SD cut-offs derived from the De Onis et al (2006) dataset. The mode and median time to walk was 12 months. Only one participant was outside 2.5 SD of the norms.

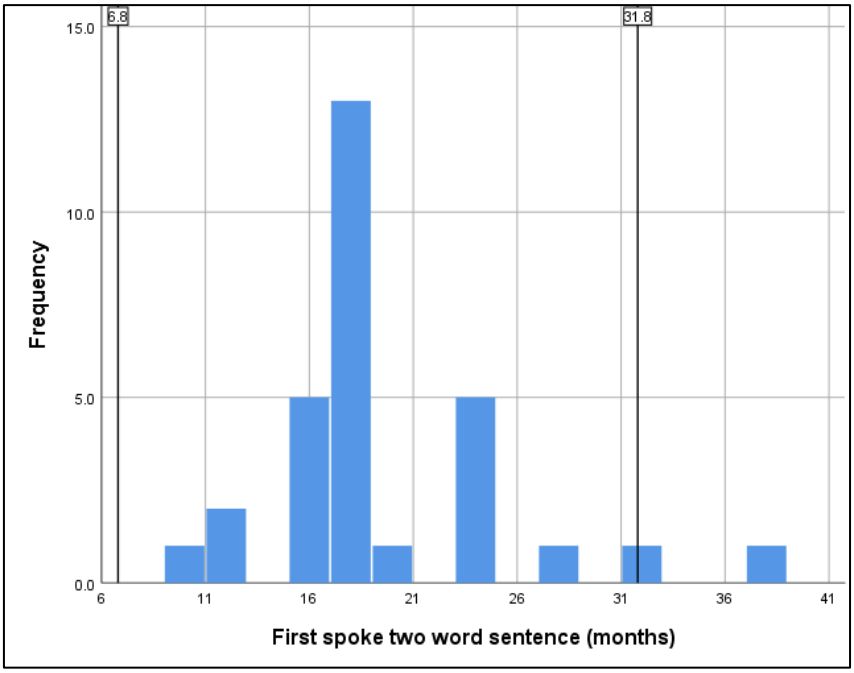


Figure 23: Age of children with RE when they first spoke a two-word sentence. Vertical lines are 2.5 SD cut-offs derived from Rudolph and Lennard (2006). The mode time to speak was 17 months. One participant was borderline abnormal in the delay to speak and another was extremely delayed.

5.3.1.4 *Developmental milestones and relationship with DCD in RE*

The RE group was divided into those with an indication with DCD and those without. The milestones of these groups were compared, and there was no significant difference between the groups for either milestone. In the group with RE and DCD, the mean time till walking without assistance was 12.5±2.44 months compared to 11.18±1.44 months in the RE non-DCD group (p=0.075). In the group with RE and DCD, the mean time to speak two-word sentences was 18.31±4.73 months in the DCD group and 20.38±6.73 months in the non-DCD group (p=0.341). The DCDQ'07 scores for both non-DCD and indication for the DCD group were compared with milestones and epilepsy metrics.

In those with DCD, there were no significant correlations between the DCDQ'07 score and age of learning to walk (r=-0.132, p=0.640), age first spoke two-word sentences (r=-1.93, p=0.527), age of first seizure (r=.001, p=.997) or duration of epilepsy (r=-1.19, p=0.672). Similarly, there were no significant correlations in the non-DCD group.

5.3.1.5 *Cognitive measures and their co-occurrence with DCD*

Test	RE	Controls	Significant difference (p=)	DF	Effect size (d=)
TOWRE:	N=22	N=19			
Sight Word Efficiency	97.45±13.1	100.84±14.5	0.439	40	0.25
Phonemic Decoding Efficiency	102.18±13.46	107.53±9.99	0.154	40	0.45
SCAN:	N=32	N=21			
Filtered Words	85.00±15.6	97.38±12.5	0.004 [a]	52	0.88
Auditory figure Ground	90.94±14.7	89.29±11.3	0.665	52	0.12
Competing Words	85.94±18.1	81.67±19.8	0.421	52	0.22
Competing Sentences	93.44±19.0	95.71±18.1	0.663	52	0.12
Connors:	N=29	N=21			
Inattentive	51.86±10.2	50.19±9.9	0.563	49	0.17
Hyperactive	54.19±11.26	48.71±6.5	0.037	49	0.60

Table 31: Baseline cognitive scores in individuals with RE and controls.

Scores for each test include the mean and standard deviation. Statistics calculated using a t-test. DF = Degrees of freedom. [a] Survived Bonferroni correction p=0.05. Cohens D used to calculate effect sizes. A significant difference between the groups was found for filtered words and hyperactivity, but only the filtered word deficit survived Bonferroni correction.

Presented in Table 31 are the group results for TOWRE, SCAN and Connors Questionnaires. Significant differences were found for the filtered words subtest of SCAN and the DSM-IV hyperactive score of the Connors CBRS with large to moderate effect sizes, respectively. It is important to note that the filtered word's score was 1 SD below normal, whereas the mean hyperactivity score was

close to normal. All the other tests were nonsignificant. The RE group was then split into those who indicated DCD and those who did not, and a comparison between test results was made.

Test	DCD +	DCD -	Significant difference (p=)	DF	Effect size (d=)
TOWRE:	N=11	N=10			
Sight Word Efficiency	92.82±14.9	101±9.9	0.127	20	0.65
Phonemic Decoding Efficiency	100.1±13	105±14	0.421	20	0.36
SCAN:	N=15	N=16			
Filtered Words	86.33±15.71	83.75±16.5	0.659	30	0.16
Auditory figure Ground	90±14.8	91.56±15.6	0.776	30	0.10
Competing Words	87.67±17.3	86.25±17.2	0.826	30	0.08
Competing Sentences	89.67±17.3	96.88±20.96	0.304	30	0.38
Connors:	N=29	N=21			
Inattentive	52.33±10.1	51.54±11.08	0.845	49	0.08
Hyperactive	55.00±10.9	53.08±13.09	0.679	49	0.16

Table 32: Cognitive scores for individuals with RE with or without an indication for DCD at baseline. DCD+: With an indication for DCD, DCD-: Without an indication for DCD. Included were t-test significant differences and effect sizes using Cohens d. DF = Degrees of freedom. The division of the RE group into those with an indication for DCD and those without an indication did not reveal a statistical difference in cognitive scores.

The RE group data was then split into those with DCD and those without to see if indicating DCD influence other cognitive functions at baseline Table 32. There was no significant difference between these groups; however, a moderate effect size was seen for the sight word efficiency task.

5.3.1.6 *Indication for an SPLD in the RE and control groups*

Moderate	N RE (%)	N Controls (%)	Odds ratio (95% CI)	DF	Significance (p=)
RD	6 (27.7)	3 (15.8)	2.0 (0.42-9.41)	40	0.38
CAPD	8 (25.0)	7 (33.3)	0.66 (0.2-2.2)	52	0.51
ADHD	4 (13.8)	1 (9.0)	3.36 (0.34-32.4)	49	0.29
Severe	RE (%)	Controls (%)	Odds ratio (95% CI)		Significance
RD	0	0	N/A	40	N/A
CAPD	5(15)	1(4.76)	3.70 (0.40-34.2)	52	0.25
ADHD	2(6.9)	1(4.5)	1.55 (0.13-18.3)	49	0.72

Table 33: Indication for SPLD in the RE and control groups at baseline. Moderate 1 SD below the mean and severe 2 SD below the mean. Statistical analysis using χ^2 with 95% confidence intervals. When the SPLD measures were stratified into moderate and severe, there were no significant differences between the RE group and healthy controls at baseline.

The participants in both the RE and control groups were then divided into those with that fulfilled an indication for each SPLD which include developmental dyslexia (DD), central auditory processing disorder (CAPD) and attention deficit hyperactivity disorder (ADHD). The classification used the cut-off scores as defined in the methods section. The data is presented in Table 33; there are no significant differences between the groups, but severe impairments were more prevalent in the RE group. In the moderate indication group, individuals with RE were two times more likely to have RD and three times more likely to have ADHD. In the severe group, individuals with RE were 3.7 more likely to have a CAPD and 1.5 more likely to have ADHD. Interestingly, evidence for the indication of severe developmental dyslexia was not apparent in either group at baseline.

The occurrence of indication for each of the SPLDs was plotted in Venn diagrams to visualise the extent of overlap. Only individuals with a complete, TOWRE-2, DCDQ'07 and Connors questionnaires were included in the analysis. Figure 24 shows in the RE group; it is common to have more than one SPLD. DCD dominates the diagram, and this appears to be strongly associated with RD, ADHD is the least apparent SPLD. Interestingly there was evidence of co-occurring RD and ADHD. The same relationship was seen in the control group. In the control group, SPLD traits are infrequent, and there is a complete separation between having an indication for DCD and ADHD and RD. DCD and ADHD appeared to co-occur whereas an indication for RD despite being as common as DCD, was separate.

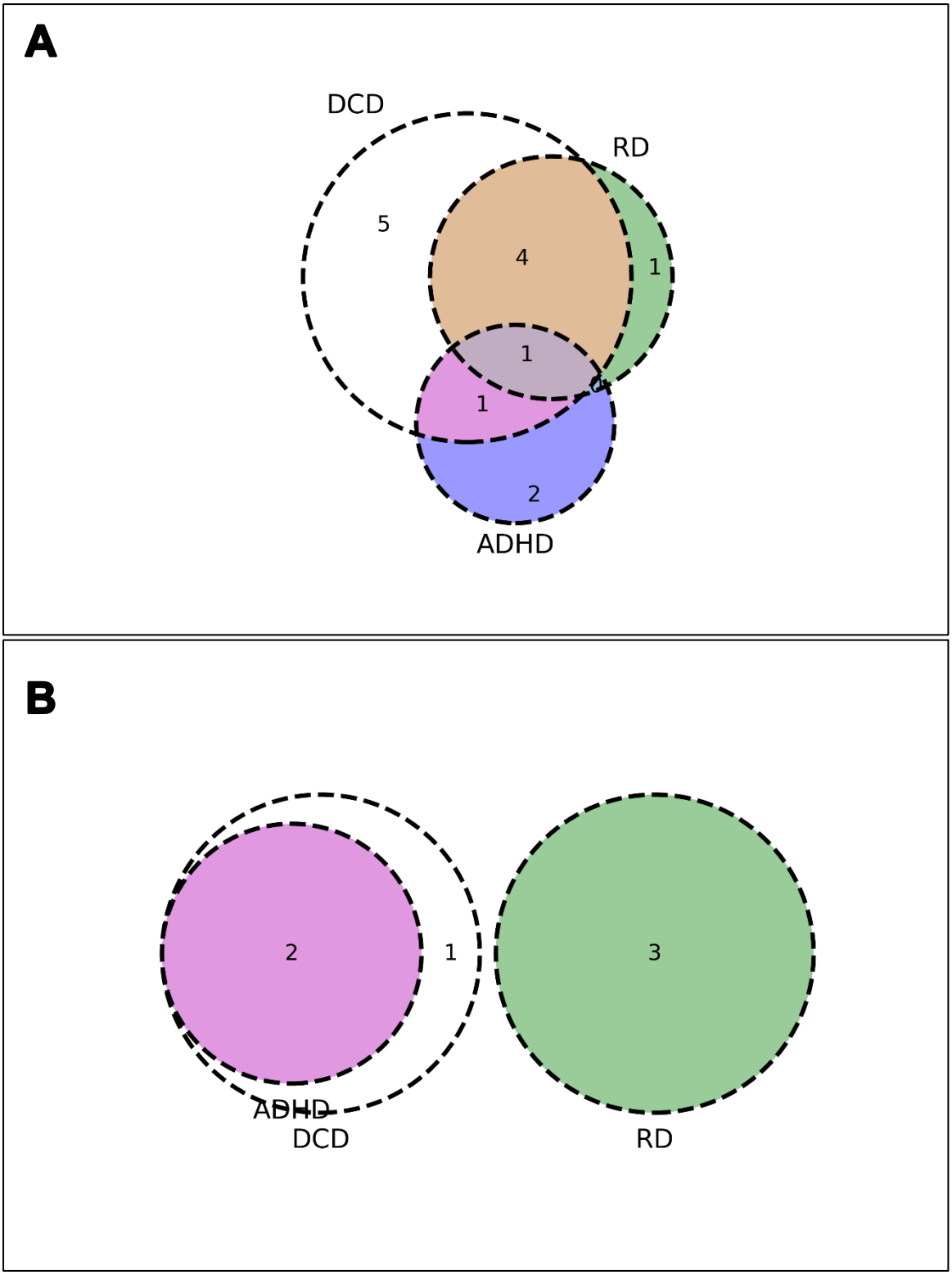


Figure 24: Overlaps between indication for DCD and traits for SPLDs in individuals with RE and healthy controls at baseline. Presented are only those participants who had an indication for a SPLD. A: Children with RE, B: Healthy controls. DCD (white), RD (green) and ADHD (purple). Colours alter in areas of overlap. In the RE cohort there are many participants with an indication for SPLDs. The most commonly occurring is an indication for DCD. In the control group SPLDs are less prevalent. Moreover, there is reduced overlap with DCD and ADHD segregated from dyslexia.

5.3.2 **Follow-up cohort**

At follow-up 17 adolescents with RE (47.2% of baseline) and 16 controls (72.7% of baseline) returned. The participants were followed up within 4.94±0.8 years of the baseline measurements. The table below contains the demographics in the follow-up cohorts.

	RE (n=17)	Controls (n=16)	DF	Significance (p=)
Mean age (years ±1SD)	14.68±2.65	17.05±2.46	32	0.012
Sex (% male)	64.7	50	32	0.39
Right-handed (%)	94.1	100	32	0.33
First seizure (years)	7.17±2.09			
Final seizure (years)	12.23±2.01			
Duration of epilepsy (years)	5.06±2.18			
AED use at follow-up (%)	37.5			

Table 34: Follow-up demographics for individuals with RE in seizure remission and controls. Statistical comparisons of percentages calculated using χ^2. Statistical comparison of age by t-test. DF = Degrees of freedom. At follow-up, there was a significant difference in age between the RE and healthy control group.

5.3.2.1 *Indication for DCD in seizure remission.*

There was no significant difference for an indication for DCD between the follow-up cohorts (p=0.48). Five individuals with RE (29.4%) indicated for DCD compared to 3 (18.75%) in the control group, odds ratio (1.8, CI 0.35 to 9.24). A comparison of DCDQ'07 scores between the RE group (62.88±10.06) and the controls (66.25±7.41) was found to be not significant (p= 0.281).

Test	RE	Controls	DF	Significance (p=)	Effect size (d=)
TOWRE:	n=12	n=13			
Sight Word Efficiency	97.33±13.8	99±7.78	24	0.716	0.14
Phonemic Decoding Efficiency	101.17±13.82	105.71±10.8	24	0.367	0.36
SCAN:	n=13	n=14			
Filtered Words	96.29±10.27	95.47±12.49	26	0.853	0.07
Auditory figure Ground	84.57±9.59	79.07±18.79	26	0.345	0.49
Competing Words	87.42±19.41	100.78±16.79	26	0.069	0.73
Competing Sentences	95.88±18.23	100.32±18.03	26	0.530	0.24
Connors:	n=17	n=16			
Inattentive	52.7±12.98	49.75±9.99	32	0.468	0.25
Hyperactive	50.47±12.40	49.69±9.69	32	0.841	0.07

Table 35: Comparison of SPLD indicator scores in individuals with RE in seizure remission and controls. Included are the statistical significance between the groups calculated by -t-test and effect size Cohens d. There was no significant difference between the group at baseline other than an approach towards significance in the competing words task.

Indeed, no significant statistical difference could be found between the two groups. Despite this moderate effect sizes were seen for auditory figure-ground and competing words. The cut-off results revealed a similar picture.

Moderate	RE (%)	Controls (%)	DF	Odds ratio (95% CI)	Significance (p=)
RD	4 (33.3)	0(0)	24	14.29 (0.68-300.38)	0.086
CAPD	2 (15.4)	2 (14.3)	26	1.09 (0.13-9.12)	0.94
ADHD	3 (17.6)	4 (25)	32	0.64 (0.12-3.46)	0.60
Severe	RE (%)	Controls (%)		Odds ratio (95% CI)	Significance
RD	0 (0)	0 (0)	24	N/A	N/A
CAPD	2 (15.38)	1(7.14)	26	2.36 (0.18-29.7)	0.5
ADHD	1 (5.9)	0 (0)	32	3 (0.1-79.1)	0.51

Table 36: Indication for SPLD in individuals with RE in seizure remission and controls at follow-up. Statistical comparisons between the percentage for an indication of an SPLD by χ^2. DF = Degrees of freedom. Cut-offs for moderate and severe SPLDs revealed no statistically significant difference between the RE and healthy control groups.

The cut-offs demonstrated no significant differences between the groups. Nevertheless, moderate RD was the closest towards significance, and there appeared to be more individuals with severe indications for CAPD and ADHD in the RE group.

5.3.2.2 *Detailed cognitive assessment in seizure remission*

Several other tests were used to assess central auditory processing, fluid intelligence, phonological function and motor skills (Table 37).

Test	RE	Controls	DF	Significant difference (p =)	Effect size (d)
WASI	N=13	N=13			
Matrices'	100.42±10.8	109.14±8	25	0.023 [a]	0.92
SCAN-3	N=13	N=13			
Gap detection failed (%)	3 (23%)	0 (0%)	25	0.029 [a]	n/a
Time compressed sentences	85.4±17.5	89.6±25.1	25	0.620	0.2
CTOPP	N=13	N=14			
Blending Words	93.98±13.39	102.08±14.5	26	0.144	0.58
Phoneme Isolation	99.23±12.69	102.71±9.80	26	0.436	0.31
Memory for digits	98.84±13.09	110.71±14.39	26	0.034 [a]	0.86
Non word repetition	90.75±16.02	103.75±16.16	26	0.046 [a]	0.80
Rapid digit naming	92.96±15.6	90.49±16.76	26	0.695	0.15
Rapid letter naming	86.8±15.8	93.7±16.01	26	0.275	0.43

Blending non-words	97.21±12.6	105.35±12.19	26	0.101	0.66
Segmenting non-words	92.3±14.26	100.84±12.80	26	0.117	0.63
Grooved Pegboard	N=14	N=14			
Dominant hand	84.2±27.55	101.84±13.41	27	0.044 [a]	0.81
Non-dominant hand	81.3±31.85	101.38±8.74	27	0.038 [a]	0.86
DASH	N=14	N=14			
Copy best	94.46±17.08	100.69±12.53	27	0.281	0.42
Copy Fast	91.16±20.98	98.13±12.03	27	0.293	0.41
Alphabet	94.19±15.41	98.99±13.04	27	0.382	0.34
Free Writing	94.53±11.53	96.54±13.55	27	0.677	0.16
Graphic speed (both groups n=13)	86.77±13.65	93.19±15.16	27	0.288	0.45

Table 37: Follow-up additional cognitive task scores in individuals with RE in seizure remission and controls. All of the results were statistically analysed using a t-test except for the gap detection test, which was categorical and was analysed using the χ^2 test. DF = Degrees of freedom. [a] did not survive Bonferroni correction. At follow-up, a significant difference was found for matrices performance, gap detection, memory for digits, non-repetition and dominant and non-dominant hand performance using the grooved pegboard demonstrating difficulties for individuals with RE. None of these findings survived Bonferroni correction.

The following tests results were significant; WASI matrices, Gap detection, memory for digits, non-word repetition and pegboard scores in both the dominant and non-dominant hands. None of these findings survived correction for multiple comparisons; nevertheless, all of these significant results had large effect sizes which strengthen their interpretation. It is important to note that the matrices scores and memory for digits scores were close to normal standard scores whereas the group non-word repetition was bordering a reduction of 1SD and the pegboard scores were less than 1SD indicating the greatest impairment.

5.3.3 **Longitudinal changes**

In this section, the changes between baseline and follow-up time points within participants are presented. To check for bias in the attrition of participants an analysis was performed. This analysis investigated the difference in WASI, DCDQ'07, TOWRE, SCAN and CBRS scores derived at baseline between those participants who returned for follow-up and those who were lost to attrition. Both the RE group and healthy control group were assessed using MANCOVA analysis corrected for sex and handedness. In the RE group, there was no significant difference ($p = 0.838$) between the five lost to attrition and the thirteen returnees. In the control group, there was no significant difference ($p = 0.767$) between the twelve returning participants and seven lost to attrition. These findings would suggest that drop out in the study was a random process.

5.3.3.1 *Intelligence and reasoning*

The matrix reasoning test was used to assess the groups for their fluid intelligence and reasoning abilities (Figure 25).

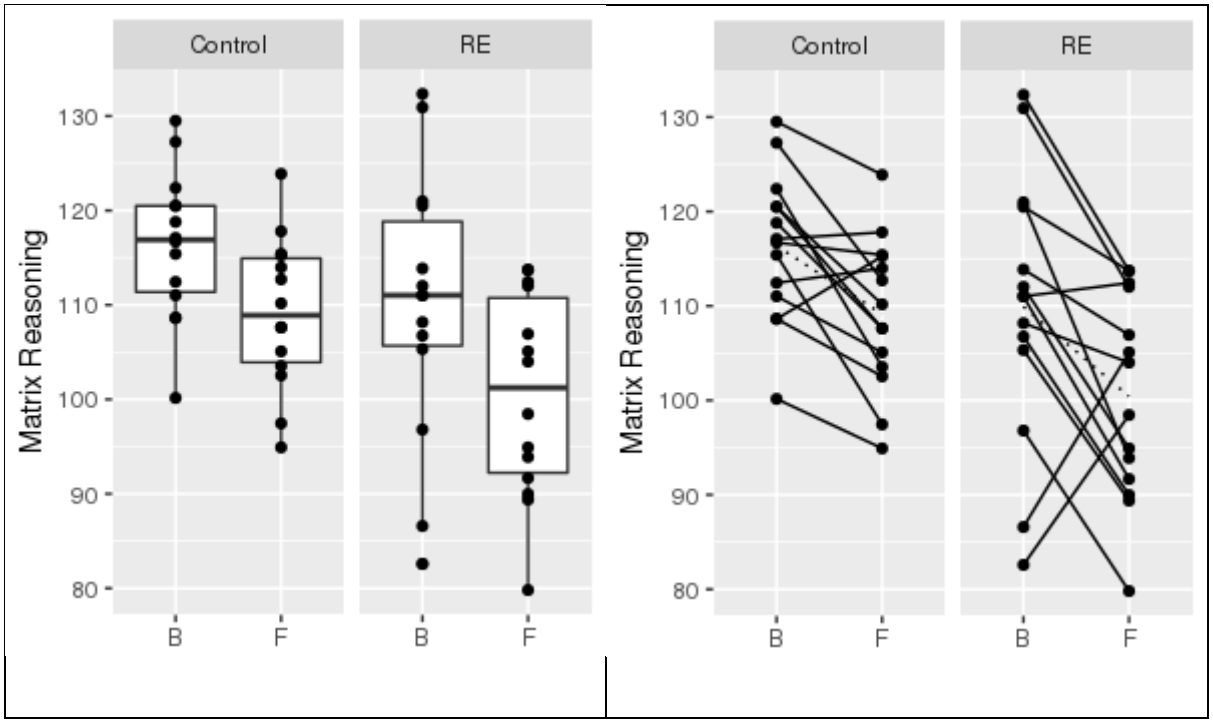


Figure 25: Longitudinal matrix reasoning scores in individuals with RE and controls. Left: Boxplots, Right: Individual change (black lines) and mean change (dotted black line) B= Baseline, F = Follow-up. A decrease in matrix reasoning scores are seen in both the control and the RE group between baseline and follow-up. The reduction in scores is greatest in the RE group, as many of the participants drop below the 100 average mark.

In both the control and the RE group, there was a decrease in scores for matrix reasoning. Unlike the control group, the majority of the RE participants had a large decrease in score with most scoring under 100 in the follow-up. The linear mixed effect (LME) model found a significant difference in change ($p=0.028$, χ^2 [1] = 417.99) between the groups. It calculated a lowering WASI matrices scores in those with RE by -7.866±3.58 standard errors (SE) between the time points. Overall, the findings suggest a worsening in fluid intelligence in the RE group in seizure remission.

5.3.3.2 *Reading ability*

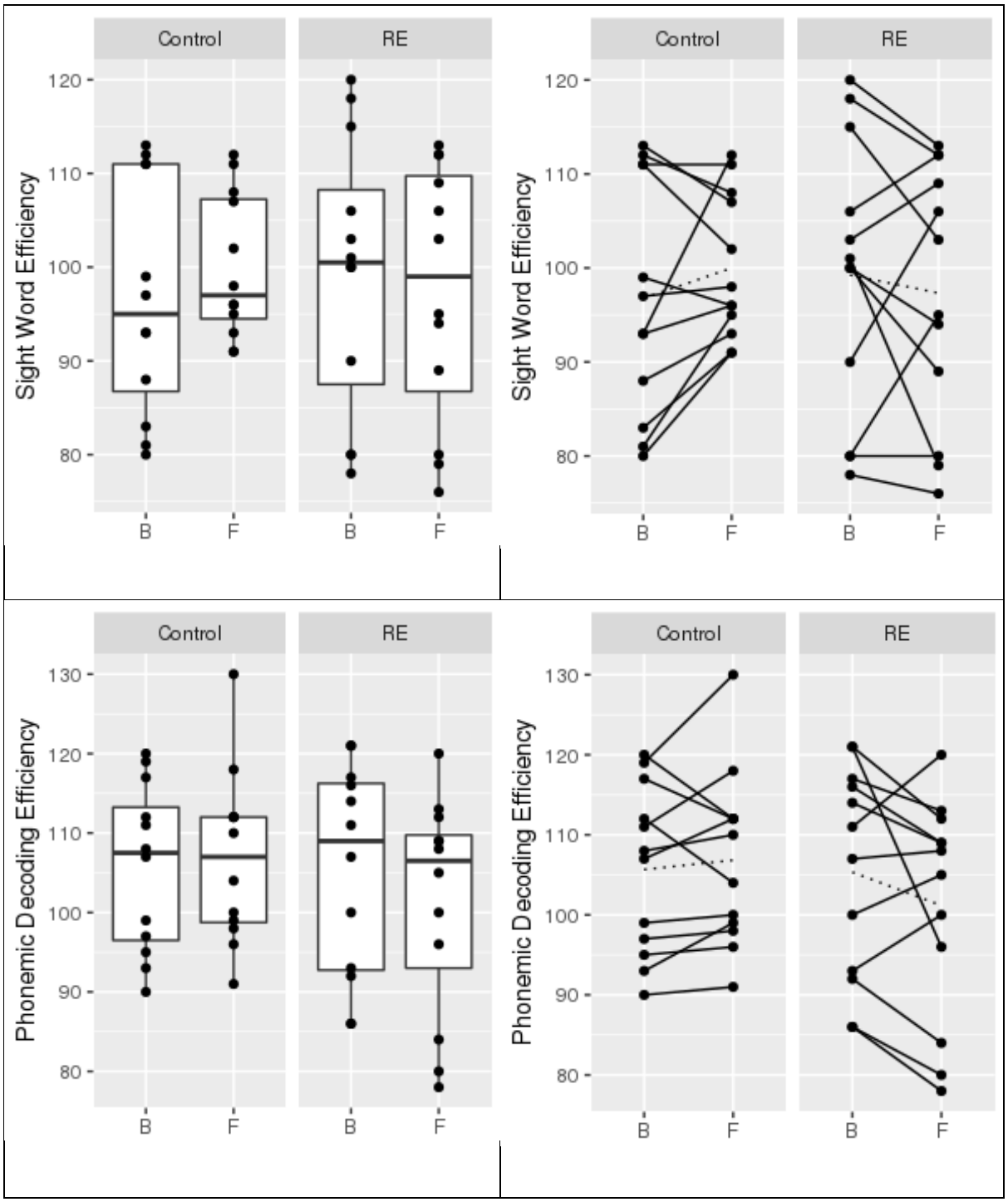


Figure 26: Longitudinal SWE and PDE scores in individuals with RE and controls.Left: Boxplots, Right: Individual change (black lines) and mean change (dotted black line) B= Baseline, F = Follow-up. As a group, the control participants improved in sight word efficiency (SWE) and phonemic decoding efficiency (PDE). The group mean in the controls demonstrates an improvement over time in SWE and PDE whereas in the RE group performance decreases between baseline and follow-up.

Reading ability was assessed using the TOWRE-2 test, which assessed sight word reading (SWE) and phonemic decoding (PDE). LME analysis revealed no significant difference in change between the groups in either SWE ($p=0.9307$, χ^2 [1] =0.0076) or PDE ($p=0.5415$, χ^2 [1] = 0.37) tests. Despite this, the mean change in the RE group was for the SWE and PDE scores to decrease over time in whereas in the control group they increased. In the RE group, scores of SWE and PDE were more likely to decrease below 100. Overall, the findings indicate little change in lexical and non-lexical reading ability in both groups between baseline and follow-up.

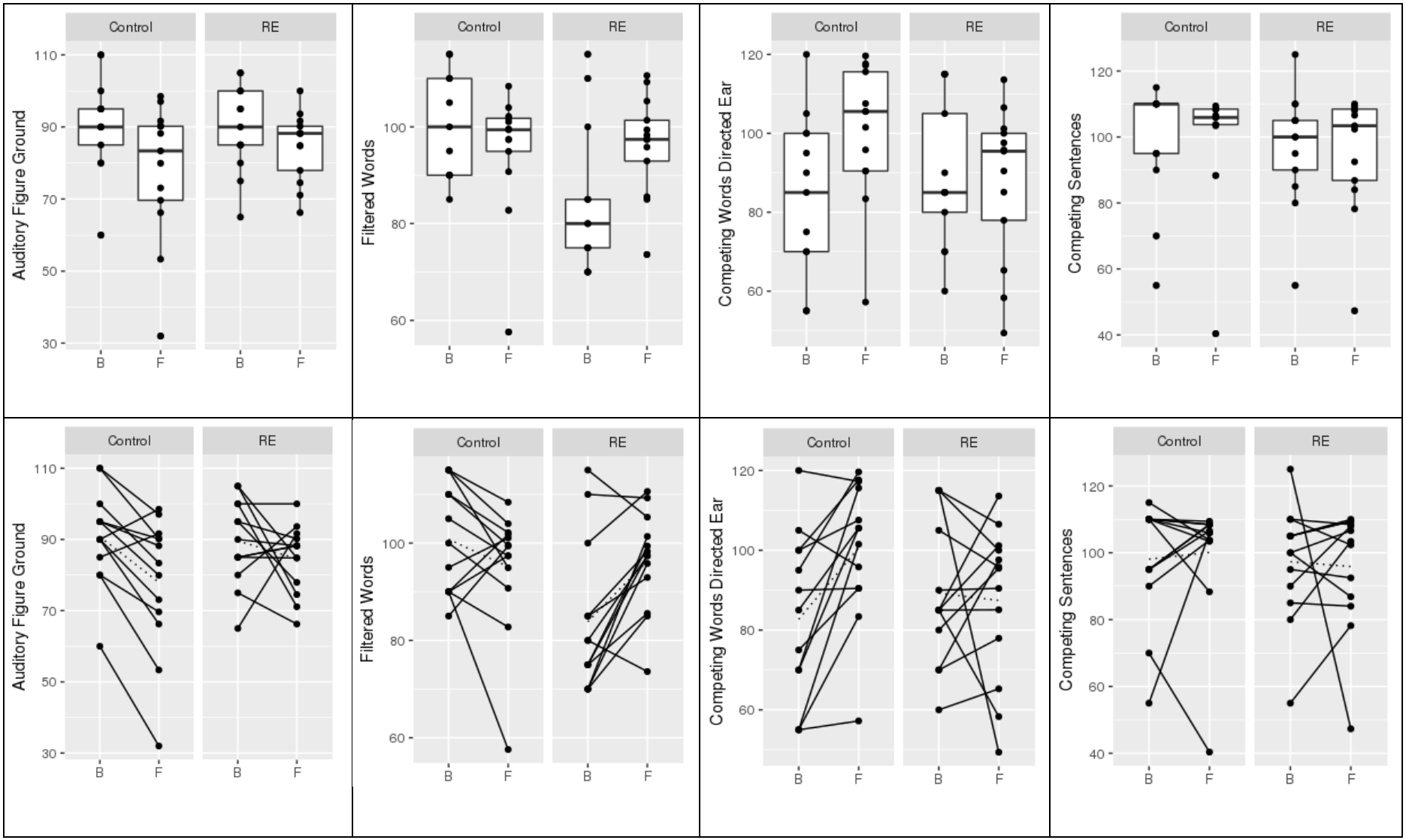


Figure 27: Longitudinal SCAN scores for individuals with RE and controls. Top row: Boxplots. Lower row: Individual change (black lines) and mean change (dotted black line) B= Baseline, F = Follow-up. In the control group performance in the auditory figure-ground and filtered words, tasks became worse between the baseline and follow-up

whereas in both dichotic tasks there was an improvement. In the RE group, the only clear improvement was in the filtered words task whereas there was a decrease in the group mean in the auditory ground and both dichotic listening tasks.

5.3.3.3 *Central auditory processing disorders*

Central auditory processing was assessed using the four components of the SCAN test; auditory figure-ground, filtered words, competing words directed ear and competing sentences (Figure 27). The LME model found a significant difference, in the filtered words task, mean score differed slightly between the baseline and follow-up in the control group. LME analysis found a significant difference between the groups (p=0.045, χ^2 [1] = 411.28). The improvement in filtered words scores equated to an 8.47±4.07 (SE) increase in filtered words scores in the baseline group between baseline and follow-up. All the other, tests were non-significant, this included the auditory figure-ground (p=0.75, χ^2 [1] = 412.89), competing words (p=0.44, χ^2 [1] = 453.15) and competing sentences (p=0.62, χ^2 [1] = 443.44). Despite the lack of significance, there was variation in mean change between the groups, the improvement in competing words scores seen in the control group was not seen in the RE group. Overall, the statistics would suggest an improvement in the auditory processing of filtered words in RE in seizure remission.

5.3.3.4 *Developmental coordination disorder*

The participants were assessed for an indication for DCD using the DCDQ'07 questionnaire (Figure 28).

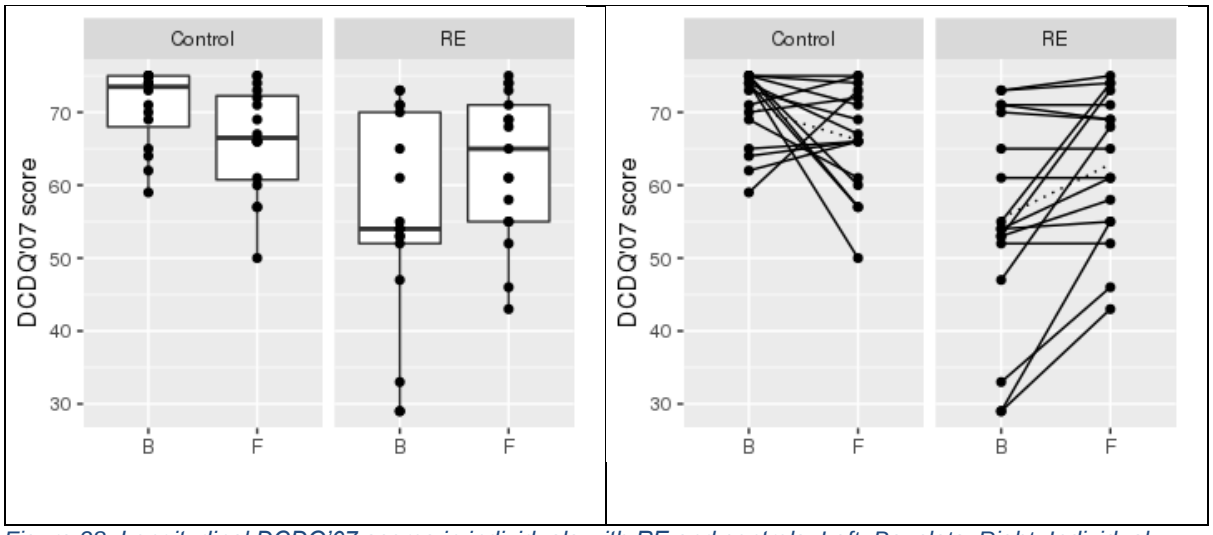


Figure 28: Longitudinal DCDQ'07 scores in individuals with RE and controls. Left: Boxplots, Right: Individual change (black lines) and mean change (dotted black line) B= Baseline, F = Follow-up. In the control group, DCDQ'07 scores reduced between baseline and follow-up, whereas in the RE group there was a large improvement in DCDQ'07 scores.

LME statistical analysis found LME analysis found a significant difference between the groups ($p=0.005$, χ^2 [1] = 483.61). The model identified between the two time-points an increase in the score in the RE group by 8.26±2.8 (SE). These findings would suggest a significant improvement in DCDQ'07 scores in the RE group in seizure remission.

5.3.3.5 *Attention deficit hyperactivity disorder*

The participants were assessed using the Connors Behaviour Ratings Scales questionnaire, which provides a score to aid in the diagnosis of DSM-IV hyperactive and inattentive subtypes of ADHD (Figure 29).

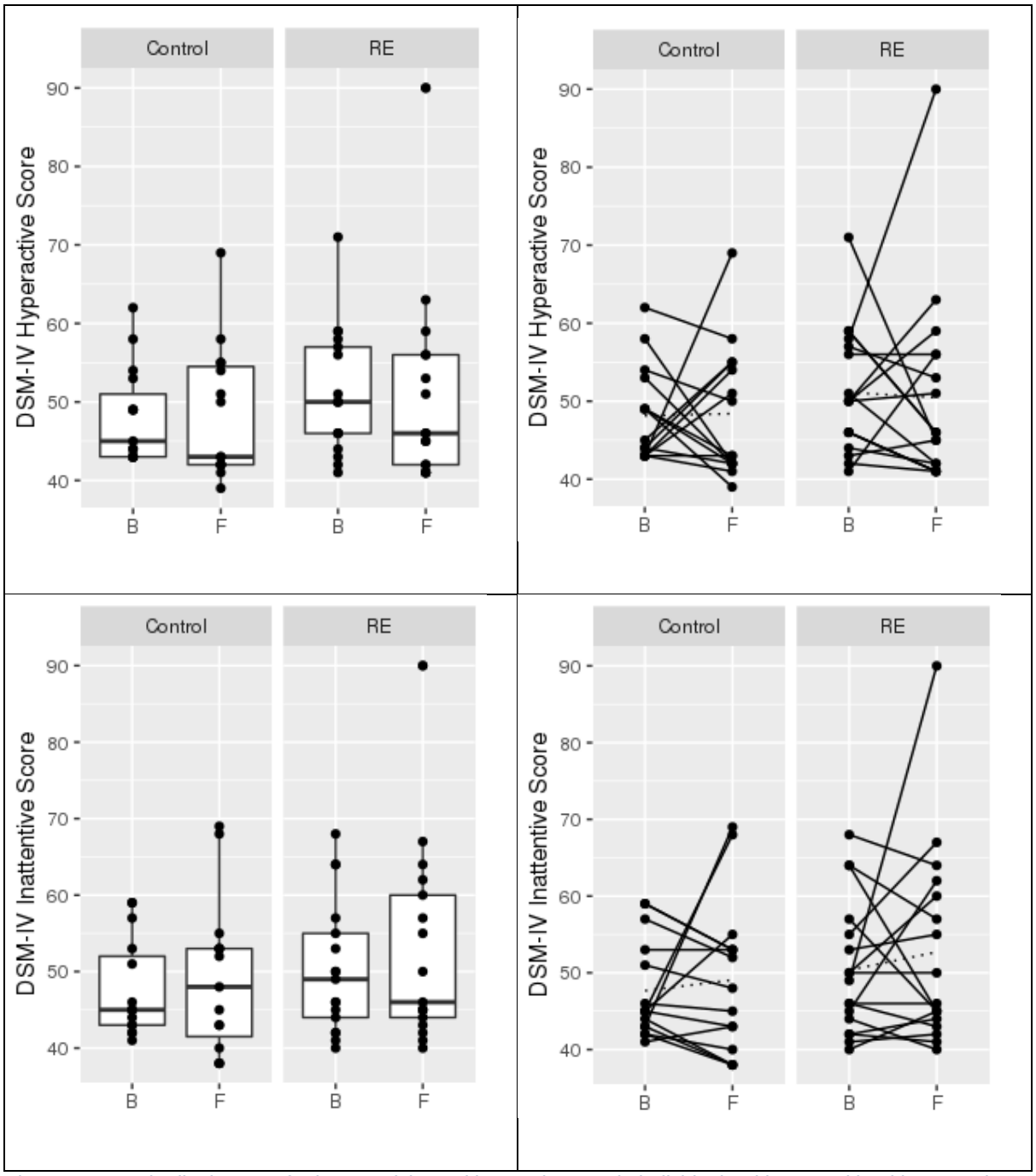


Figure 29: Longitudinal scores for hyperactivity and inattentiveness in individuals with RE and healthy controls. Left: Boxplots, Right: Individual change (black lines) and mean change (dotted black line) B= Baseline, F = Follow-up. In the measurement of hyperactivity, other than elevated scores in the RE group, there was little difference in the change between the control and RE groups between baseline and follow-up. In the

measurement of inattentiveness, both the control and the RE group had an elevation of scores in the follow-up; this was greater in the RE group.

LME analysis found there was no significant difference between the groups in both hyperactivity (p=0.29, χ^2 [1] = 460.17) and inattentive scores (p=0.31, χ^2 [1] = 466.53). In sum, these findings would suggest little change in group scores between the time-points.

5.3.3.6 *Individual co-occurring cognitive problems and change*

Participant	B-DCD	F-DCD	B-RD	F-RD	B-ADHD	F-ADHD	B-SPLD freq	F-SPLD freq
1								
2				SWE/PDE	In			
3			SWE			In		
4								
5				SWE				
6			SWE	SWE/PDE	In	In		
7								
8								
9			SWE	SWE				
10								
11								
12								
13					In			
14			SWE					
15						In/Hyp		
Total (%)	60	26.6	26.6	26.6	20	20	66.6	46.6

Table 38: Impairment in individuals with RE at baseline and follow-upFifteen participants with RE were classified as having an indication for DCD: Developmental coordination disorder, RD: Reading disability and ADHD: Attention Deficit Hyperactivity Disorder at baseline (B) and follow-up (F). Light yellow denotes baseline only impairment; orange denotes the deficit was recorded at baseline and follow-up, and the deficit only recorded at follow-up (bright yellow). B-SPLD freq: Number of SPLDs per participant at baseline, F-SPLD: Number of SPLDs per participant at follow-up. Grey: No SPLD's. Light blue squares one SPLD, Blue square: Two SPLDs, Dark blue: 3 SPLDs.

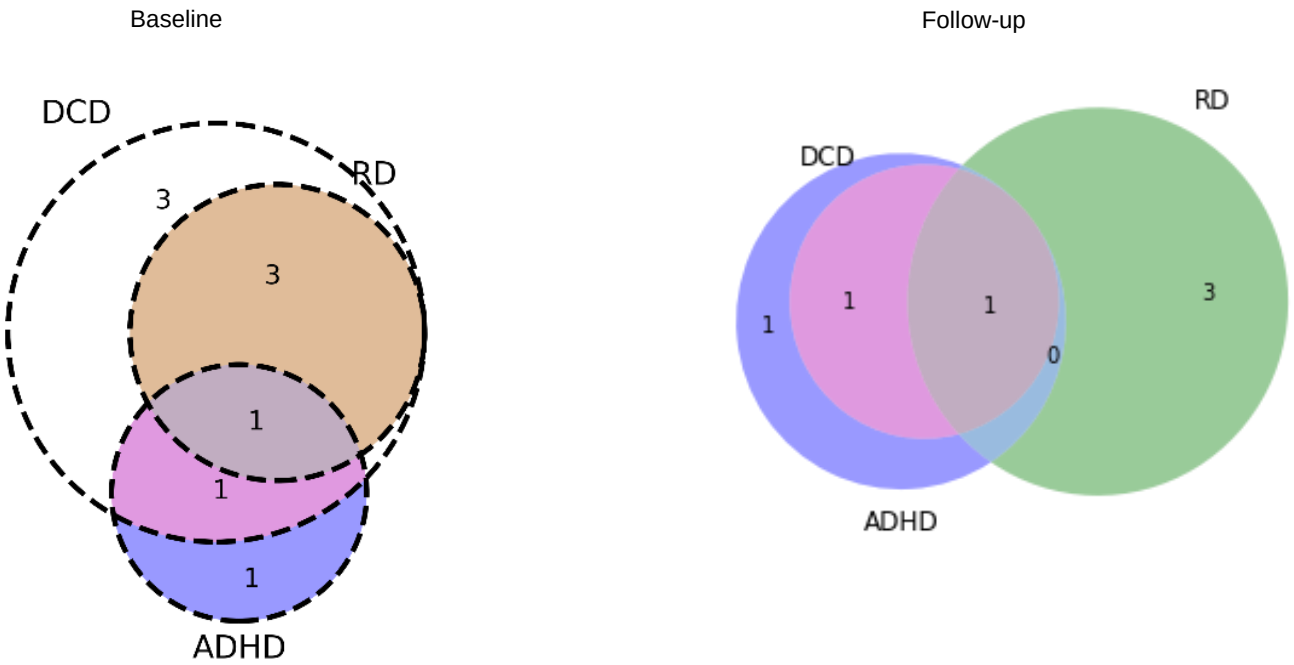


Figure 30: Longitudinal inter-relationships between cognitive deficits in individuals with RE between active epilepsy and seizure remission. Only participants with an indication for an SPLD are included. Baseline: DCD (white), RD (orange) and ADHD (purple). Colours alter in areas of overlap. Follow-up: DCD (purple), ADHD (pink) and RD (green). At baseline, there is evidence of a large overlap between SPLDs with an indication for DCD being the most prominent. Whereas, at follow-up there is a separation between participants with DCD and those with RD.

The longitudinal data were classified and sorted into groups, to explore overlaps between cognitive problems. Fifteen participants had full longitudinal data for classification. At baseline ten (66.7%) had one or more impairments, and at follow-up, this reduced to 7 (46.6%) the same number were impaired. Over both time points, only three participants (20%) did not indicate an SPLD. Interestingly, there was a mixture of persisting deficits and deficits which appeared at follow-up (Table 38).

The individual data shows that SPLDs detected at baseline can persist into seizure remission (Table 38). DCD was the most prevalent SPLD in both the baseline (60%) and follow-up results (26.6%). Indeed, only those individuals with DCD at baseline would have an indication for DCD at follow-up. Other SPLDs seen in individuals in both baseline and follow-up included RD (13.3%) and ADHD (6.6%). In RD, those who had a deficit in lexical reading had this persist into follow-up without additional non-lexical impairment. In ADHD, the inattentive sub-type would persist into seizure remission. Overall, the findings reveal DCD to be the most common and persistent SPLD where RD and ADHD were less frequent and would be less likely to persist. Some individuals only developed SPLDs in the follow-up.

There was evidence of new RD and ADHD but not DCD in seizure remission. New SPLDs occurred in four individuals (26.6%); half of this group had no evidence of SPLD at baseline. In the follow-up, two individuals had new RD (13.3%), and two had ADHD (13.3%). New RD was characterised by problems in either lexical or non-lexical reading, and new ADHD was predominantly inattentive. The new RD problems were seen on their own, whereas the ADHD problems could be either independent or co-occur with DCD. Analysis of both Table 38 and Figure 26 reveals frequent overlaps of SPLDS in the baseline, which was reduced in the follow-up.

The occurrence of two or more SPLDs was common. At baseline, five individuals (33.3%) had two or more co-occurring SPLDs, whereas, at follow-up, this reduced to two individuals (13%). This diagram shows that in individuals with DCD and RE had the most occurring problems at baseline and follow-up. The RD SDLP appears to not occur with other disorders in the follow-up, which is of interest as it mirrors the distribution of SPLDs in the baseline control cohort (Figure 24).

The individual data created from cut-offs would suggest that over both time-points having an indication for an SPLD is the norm and not the exception. DCD in the RE in the most common co-occurring SPLD and this can persist into seizure remission. Furthermore, it can frequently occur with

other SPLDs such as RD and ADHD. Finally, new evidence for SPLDs can become apparent in seizure remission, this includes RD and ADHD, and these can be become apparent without or without the influence of other SPLDs in the individual's history.

5.4 Conclusions

This study was designed to see what the prevalence of DCD in individuals with RE and how this co-occurs with other SPLDs. The second objective was to identify the change in DCD and other co-occurring SPLDs between active epilepsy and seizure remission. This section will be divided up into cross-sectional, longitudinal, limitations and implications.

5.4.1 Cross-sectional

5.4.1.1 *Developmental coordination disorder is prevalent in children with RE compared to controls*

The findings of this study would suggest that an indication for DCD is the most prevalent co-occurring cognitive problem in individuals with RE. Around 41.7% of children with RE will indicate DCD, and this is 4.75 times more likely than the control population. An increased prevalence for DCD is in agreement with the work of Kirby *et al*., who found a similar prevalence in their cohort (Kirby *et al.*, 2017). In seizure remission, 29.4% of the follow-up cohort indicated for DCD. However, the odds ratio compared to controls was reduced compared to the baseline cohort. Evidence for an indication for DCD in seizure remission has not been reported in the literature.

5.4.1.2 *No association between delayed milestones and developmental coordination disorder*

Despite a high prevalence of DCD, there is little evidence to associate delayed development with cognitive disorder. This study would suggest that there is no association between a delay to speak two-word sentences or walk up aided. Indeed, the milestones of individuals with RE mostly fit within the distribution of normal data. However, a few outliers were seen in both delay to walk (2.7%), and

delayed speech (6.6%) and this warrants further investigation as it could be an indication of a sub-type. The lack of evidence for developmental delay does not agree with those in the literature.

Overvliet *et al.*, found that 25% had a motor delay, and 29.2% had a delay in language development (G. M. Overvliet *et al.*, 2011). There is a stark difference with this studies results, but it is important to note the methodology used by Overvliet et al. They asked parents their opinion whether their child had “a delay in development” and whether they had received speech therapy. An opinion about delay can lead to an over or under-estimation furthermore; a child can attend speech therapy for many reasons other than delay. In contrast, this study asked parents about well-defined milestones and then used strict 2.5 SD cut-offs derived from large normal datasets to identify abnormal development. The lack of evidence of delay in children with RE could suggest that the onset of motor problems occurs post 36 months, which could make this form of DCD atypical.

Motor problems in typical DCD include delayed developmental milestones. Despite searching for hard evidence of delayed development in DCD, the only evidence is anecdotal. In a review by Gibbs, they found that parents frequently report delayed developmental milestones such as crawling, walking and speech with 25% of children with DCD being referred before they start mainstream schooling (Gibbs, Appleton and Appleton, 2007). This evidence would suggest that there is an atypical component to the developmental coordination disorder seen in individuals with RE.

5.4.1.3 *Individuals with RE and DCD show no difference in cognitive abilities to those without DCD*

An unexpected finding was the lack of difference in cognitive abilities between those with DCD and those without as it was hypothesised that having DCD would increase the likelihood for other SPLDS. Even so, the effect sizes were moderate for sight word efficiency, phonemic decoding efficiency and competing sentences. A search for relevant literature found nothing, which makes it difficult to identify whether this is a robust finding. Nevertheless, the finding could suggest two possibilities. One, regardless of an indication for DCD there is a risk for other cognitive problems or two, the DCDQ is not as sensitive as previously thought, and it is not detecting some individuals who have DCD which

is distorting the results. As the effect sizes for sight word efficiency, phonemic decoding efficiency and competing sentences are moderate, it would be worth repeating this study in another larger RE population and confirming DCD with the Movement ABC task.

5.4.1.4 *Central auditory processing problems and hyperactive behaviour in active epilepsy.*

At baseline, individuals with RE were significantly different in both the filtered words task and hyperactive ADHD scores. Despite not surviving Bonferroni correction, there was a moderate effect size for hyperactive ADHD in the RE group. It is important to note that as a group, this score was close to normal, which would indicate either a mild hyperactive behaviour or a heterogeneous group. Hyperactive ADHD in active epilepsy is one of the most common co-occurring cognitive problems. A recent study reported that 33% of children with epilepsy had a diagnosis of ADHD (Reilly *et al.*, 2014). Regarding evidence of ADHD in the RE population, one large study reports a high prevalence with 31% of individuals with RE had ADHD. The measurement of ADHD was based on a formal diagnosis using DSM IV and a positive response to psycho-stimulants (Tovia *et al.*, 2011). This study supports the finding of the baseline results; however, it does not define the sub-type of ADHD, so this will need further investigation. Evidence of CAPD was another impairment.

In individuals with RE, in active epilepsy, there is evidence of specific auditory processing deficit in the filtered words task, which in standard scores was 1 SD below the norm. Evidence of CAPD was expected; however, what was not expected was a large significance for filtered words subtest and no significant difference in the other tasks. In a study performed by Smith *et al.*; using the SCAN test they found the dichotic listening task, competing words, to be the only deficit with an effect size of 1SD (Smith *et al.*, 2017). The difference in their study may have been due to the use of 40 children with RE and a large control group 99 children. Nevertheless, the effect size in this study was large, and the statistical analysis survived correction for multiple comparisons, which would suggest that is a robust finding.

Overall, the evidence of increased hyperactive ADHD scores and reduced filtered word scores could be evidence of epilepsy-specific deficits. The follow-up cohort reinforces this interpretation.

5.4.1.5 *Hyperactive behaviour and impaired central auditory processing are not apparent in seizure remission.*

The difference in filtered word and hyperactivity scores in the baseline cohort of individuals with RE were not seen in the seizure remission cohort. ADHD is known to go into remission with age. In a study of 140 males with ADHD over ten years, with follow-up in adolescence or adulthood, 65% no longer meet the DSM-IV diagnostic criteria (Biederman *et al.*, 2010). This study demonstrates that a large proportion of children with ADHD have improvements as they age, and this would be a possible explanation for the loss of hyperactive features in the RE cohort in seizure remission. There is a debate about whether not fulfilling the DSM-IV criteria is a remission or just an altered presentation. Nevertheless, in the RE cohort, it is most likely a remission which may be related to the remission of seizures. Another possible development change is the loss of the specific auditory processing deficit seen in the baseline cohort.

In seizure remission, there is no evidence of a filtered words deficit in the RE group. This is of interest as it would suggest a maturation of the brain with time. Keith reported improved scores with age in normal children, and this was said to reflect the maturation of the central auditory nervous system (Keith, 2000). Therefore, it could be suggested that the loss of this deficit is evidence of normal brain maturation. The lack of a difference in hyperactive and filtered word scores at follow-up could be a sign of improvement. This improvement could be an outcome of brain maturation, and this may be part of the mechanism involved in the remission of seizure in individuals with RE.

5.4.1.6 *Additional cognitive testing in individuals with RE in seizure remission revealed several deficits related to executive functions*

In the follow-up RE group, there was evidence of difference with the controls in WASI matrices, the CTOPP memory for digits and non-word repetition, the SCAN-A gap detection task and bilateral pegboard scores. It is unclear whether the matrices and memory for digits scores are true differences, whereas the non-word repetition, gap detection and pegboard scores appear to be more robust. Nevertheless, these findings would suggest a processing problem. The non-word repetition deficit is an interesting feature as it may indicate previous developmental language disorder (DLD).

In a twin study by Bishop *et al.*, they found that poor non-word repetition scores were an indicator of previous DLD; furthermore, this was a heritable trait (Bishop, North and Donlan, 1996). Poor non-word repetition score is a curious phenomenon as it could be due to poor phonological memory, a poor understanding of phonemes or problems with articulation (Gathercole *et al.*, 1994). A recent study suggested that difficulties in non-word repetition in individuals with DLD could be due to a deficit in phonology rather than phonological short term memory or storage (Ebbels, Dockrell and van der Lely, 2012). A phonological processing problem could be the case in the RE group; however, the findings of this study point towards impairment of phonological memory as there was a large effect on the memory for digits task. Similar large effects were seen in the pegboard task.

The grooved pegboard task revealed poor motor performance in individuals with RE in seizure remission. The group deficit was greater than 1SD below the mean and appeared to be worse in the non-dominant hand compared to the dominant hand. It is important to note that despite lower scores, there was a lack of significance in the DASH handwriting tests. This is interesting because the grooved pegboard task was a novel motor task, whereas the DASH handwriting tasks is an everyday task for individuals in full-time education. These findings would suggest that fine motor control is impeded in seizure remission and this would suggest that the performance of new motor skills rather than learnt skills is a specific deficit in individuals with RE.

5.4.2 **Longitudinal**

5.4.2.1 *The majority of cognitive scores in seizure remission reveal no difference in change with controls.*

Most of the longitudinal scores revealed no difference in change with the control group in seizure remission, but no change does not necessarily mean cognitive improvement. There were no differences in change of the mean scores from lexical and non-lexical reading, auditory figure-ground (AFG), competing words and sentences, and DSM IV, hyperactive and inattentive scores. No difference was a good indication of cognitive improvement in the competing words and sentences task as the controls were seen to improve; in the other tasks, this was not the case. In the auditory figure-ground, both the controls and RE group had a deterioration in AFG test.

One possible explanation for the deterioration in the scores of the controls can be derived from the development of the test. Keith found that it was easier to obtain a high score in the AFG test using SCAN-C compared to SCAN-A (Keith, 1995). The SCAN-A is the basis for the SCAN-3 used in this study. Furthermore, the AFG test has a poor internal consistency, an alpha or 0.46 compared to the battery alpha of 0.77 (Keith, 1995). The low alpha score could suggest that the AFG test is measuring a different component of auditory processing or a non-auditory cognitive function. A contrary interpretation of both hyperactive and inattentive scores can be made.

In the control group, there was a little change in the hyperactive scores in the controls group and an increase in inattentions scores. In a comparison between baseline and follow-up ADHD score, the lack of significant change in hyperactive scores at the follow-up would suggest that there is evidence of hyperactivity in the individuals with RE. A statistical difference in ADHD scores was seen at baseline; however, it is important to note that these scores were elevated but within the normal range. Therefore, an alternative interpretation is that there are features of hyperactive ADHD, but this is unlikely to lead to a diagnosis. The same analysis can be made of the inattentive data, the mean data of both groups at baseline and follow-up is non-significant and within normal limits. Overall, in this

study, there is a large body of evidence to suggest improvements in multiple cognitive domains. There are two domains where significant improvements were seen

5.4.2.2 *Significant improvements were seen in two distinct cognitive domains*

There were significant improvements in both the change in the group DCD scores and the filtered words sub-test of the SCAN-3A. The improvement in motor skills would suggest that there is a contributing factor which is absent in seizure remission. Impaired motor function as a result of seizures is an interesting hypothesis, but there is evidence to suggest that parents may under-estimate motor difficulties in adolescents in particular in females (Timler, McIntyre and Hands, 2018). Furthermore, the DCDQ'07 does not include any questions about the acquisition of new motor skills, and the grooved pegboard scores, demonstrate residual deficits. A similar improvement is seen in filtered words scores.

There is a significant change in filtered words scores in individuals with RE in seizure remission. The filtered words test is commonly used to assess for CAPDs. The test involves the use of a monaural, low-redundancy speech sample which is distorted by using filters to restrict its frequency content. A typical listener can comprehend speech when parts of the speech spectrum are missing by cognitively filling in the gaps, which is known as auditory closure (Bellis, 2003). In individuals with CAPDs, auditory closure is not possible, and the speech stimulus is unintelligible. The significant improvement in follow-up suggests that individuals with RE demonstrate a specific CAPD feature which will improve with seizure remission. The evidence of problems with speech sounds is an interesting finding as it could be a represent a phonological processing deficit which can be seen in individuals with dyslexia, a common co-occurring cognitive problem in RE. The improvement in the auditory processing deficit may be linked to an improvement in motor skills.

5.4.2.3 *Matrix reasoning reveals problems with executive functions in seizure remission.*

The matrix reasoning test revealed a significant difference in change between baseline and follow-up, indicating a deterioration in cognition. The WASI matrices test is a measure of fluid intelligence, in Cattel-Horn-Carroll intelligence theory this is known as Gf ,it is the ability to solve novel problems which are not solvable using previously learned strategies (Carroll, 1993). Gf appears to be correlated with executive functions. An apparent worsening in the WASI matrices task could suggest a problem with executive functions when processing novel information.

Whether this is a true worsening of the cognitive ability is up for debate, it may be that the deficit has become more apparent as the individuals with RE have become older and the developmental trajectories of the RE and control group are sufficiently separated. Nevertheless, a deficit is apparent, and this could be due to the epilepsy or a familial cognitive profile.

5.4.3 **Prevalence and co-occurrence of SPLDs**

5.4.3.1 *No significant difference between groups in the occurrence of non-DCD SPLDs*

There were no significant differences between the groups in moderate and or severe reading disability, central auditory processing disorder or attention deficit hyperactivity disorder at baseline. Despite this, there were elevated odd ratios for moderate RD and ADHD and severe CAPD. These findings partially agree with the literature; one study found evidence of RD in 42% of children with RE, whereas in this study, this was reduced (Vega *et al.*, 2015). Although in the same study, there is a similar proportion of individuals with ADHD, which may suggest that TOWRE maybe not the best measurement of dyslexia in children with RE. Similarly, CAPD has been reported as prevalent in RE, using similar a methodology as this study, Boatman *et al*. found five out of seven participants (71%) were impaired in one or more auditory tests (Boatman *et al.*, 2008). Boatman found a greater prevalence of CAPD than this study, but it may be due to them including several extra tests within their battery. Nevertheless, the findings of this study would suggest that individuals with RE during active epilepsy are at risk of ADHD traits, disordered central auditory processing and to a lesser extent reading disability. In the follow-up cohort, RD is prominent.

In the follow-up, there were large odds ratios for severe ADHD, CAPD and moderate RD. Interestingly the prevalence of RD approached statistical significance. In the literature, there are two studies which suggest that literacy problems persist in seizure remission (Monjauze *et al.*, 2011; Filippini *et al.*, 2013). The Fillipini study found that 33.3% of the participants had evidence of RD, which is comparable to this study (Filippini *et al.*, 2013). Regarding the prevalence of ADHD and CAPD in seizure remission, there appears to be no previous literature. Even so, there are three studies which indicate impaired attention/executive function (Baglietto *et al.*, 2001; Garcia-Ramos *et al.*, 2015) but there is no indication of case prevalence which makes a direct comparison difficult. Overall, a follow-up cohort in seizure remission reveals evidence of moderate RD, severe CAPD and ADHD.

Longitudinal data reveals significant overlaps between SPLDs in active epilepsy, which separates in seizure remission.

5.4.3.2 *An overlap between DCD and RD is common in active epilepsy*

SPLDs in active epilepsy reveal a large overlap between DCD, RD and ADHD. The overlap was the greatest between evidence for DCD and RD, suggesting that these may have similar mechanisms. In the literature there, is a large body of evidence implicating motor problems in individuals with dyslexia and reading problems in children with DCD (Savage, 2004; Zwicker *et al.*, 2012). Interestingly, in the control group, despite containing individuals with an indication for SPLDs, they demonstrated no overlap between DCD and RD. Therefore, the overlap between DCD and RD in children with RE could be generated by a different mechanism to controls. Nevertheless, as demonstrated by Kaplan *et al.*, children with learning and attention problems can have a single deficit, but these are less prevalent than those with multiple deficits (Kaplan *et al.*, 1998). Another point of interest is the distribution of DCD, RD and ADHD are different in the Kaplan study to the results of this study, which a greater emphasis on cases with DCD and DCD+RD. Whereas in the Kaplan study, an indication for all three deficits was quite prevalent, this could suggest a different mechanism in the development of these SPLDs. This distinct distribution of SPLDs in individuals with RE changes in seizure remission.

5.4.3.3 *Seizure remission reveals a separation between developmental coordination disorder and reading disability*

In seizure remission, the overlaps between SPLDs appear to change. The number of DCD cases reduces to 26.6%, but the number of RD cases remains the same. Some of these changes are to be expected. In the literature, the prevalence of children with DCD with persistent difficulties in adolescence and young adulthood is between 30-70% suggesting that brain development can mitigate DCD symptoms (Tal Saban and Kirby, 2018). In the RE literature, there are no studies which

look at how dyslexia and ADHD symptoms change over time. Regardless, in the SPLD literature, there are longitudinal accounts. In dyslexia, a longitudinal study of 182 children found 55 children who fulfilled the criteria for RD. Interestingly, 27% met the criteria at school grade 2, and 33% only met criteria in grade 8 (Torppa *et al.*, 2015), this would suggest that dyslexia is not a stable phenomenon in childhood and late-onset dyslexia is common. A similar phenomenon was seen in this study, there was no evidence of RD in the control group at follow-up, and there was a mix of new and resolved cases in the RE group. Similarly, changes in ADHD symptoms can become different when individuals develop into adolescents and young adults.

In ADHD, the persistence of symptoms is low, and new onset of ADHD is rare. A meta-analysis of long-term outcomes of children with ADHD found persistence rates of 15 % (Faraone, Biederman and Mick, 2006). ADHD persistence increases to 60% if individuals fulfil the DSM-IVs partial remission criteria (Faraone, Biederman and Mick, 2006). In contrast to dyslexia, late-onset ADHD is rare. In a large study, 239 children without ADHD at nine years of age were followed up at 24 years. They found that only 13 (5.4%) had “onset of elevated ADHD symptoms and impairment in adolescence that was not attributable to other mental disorders” which makes this phenomenon rare (Sibley *et al.*, 2018). Whereas in this study, two participants with RE had evidence of ADHD only in follow-up, this equates to 13% and may indicate an altered mechanism behind the generation of ADHD in seizure remission.

Overall, the separation of overlap between the SPLD’s in seizure remission mostly is in keeping with the literature. The prevalence of DCD reduces in seizure remission, whereas late-onset dyslexia can appear. In addition, within the group, there is evidence of new ADHD symptoms, which is atypical in prevalence and may indicate a unique feature of RE in seizure remission.

5.4.4 **Implications**

5.4.4.1 *Layers of neuropsychological deficits*

The data produced by this study would point toward three layers of deficits; one layer is transient; the second involves potential cognitive deterioration, and the third is evidence of post epilepsy dysfunction. In active epilepsy, there is good evidence for an indication for DCD, elevated hyperactive ADHD scores and a specific auditory deficit for filtered words. All these features appear to transient because, in seizure remission, they all reduce and therefore are likely to be epilepsy specific. The second layer is a deterioration in specific executive function, which is demonstrated by a reduction in matrix reasoning scores. The third layer is possible permanent deficits, of unknown onset, which is revealed by novel complex tasks. These tasks include memory for digits, non-word repetition and the grooved pegboard task. There is also evidence of another specific auditory processing deficit, which may have been apparent in active epilepsy, where the detection of shorts gaps in noise is poor, these three layers of deficit point to similar regions of the brain.

5.4.4.2 *Deficits support fronto-parietal and striatal dysfunction*

The increased prevalence of specific cognitive deficits in active epilepsy and seizure remission indicates dysfunction in the frontal, parietal and striatal brain regions and the connections between them. The deficits in filtered words may be due to an under-development of white matter structures. A study by Schmithorst *et al.* found a strong positive relationship between ability at filtered words and fractional anisotropy in the genu of the left parietal lobe, corpus callosum and right inferior prefrontal gyrus (Schmithorst, Holland and Plante, 2011). In DCD, a resting state connectivity study in children with DCD demonstrated atypical connections between primary motor cortex, pre and inferior frontal cortices, and the striatum (McLeod *et al.*, 2014). Finally, a meta-analysis of ADHD neuroimaging studies implicates frontal and striatal brain structures (Frodl and Skokauskas, 2012). Overall, these

three deficits are evidence of a heterogeneous, neuro-developmental disorder of fronto-parietal and striatal brain regions.

5.4.4.3 *Features of SPLDs are present in active epilepsy and seizure remission*

Motor and working memory and phonological and central auditory processing dysfunctions are features of DCD, dyslexia, DLD and ADHD, all types of SPLDs (REF). This study would suggest that in individuals with RE, SPLDs are apparent in both active epilepsy and seizure remission. The existence of SPLDs requires a rethink about the role of medical intervention in this patient group. The data from this study indicate that individuals with RE should be routinely offered educational assessments with a comprehensive battery of neuropsychological tests to identify cognitive problems. These tests should focus on motor skills, executive functions, reading and phonological and auditory processing. Furthermore, the same battery of tests should be repeated in seizure remission to identify which individuals still require support.

5.5 Limitations

1.1.1 Indication not diagnosis may exaggerate the prevalence of deficits

This study would have produced robust findings if the tests had resulted in the fulfilment of the diagnostic criteria for the individual SPLDs within the Diagnostic and Statistical Manual V (American Psychiatric Association, 2013). The reliance on parent questionnaires has potentially weakened the study as direct measures of cognitive function is preferable to opinions. The use of parent completed questionnaires may also be a source of bias and the findings should agree with a demonstration of impairment in daily life as a result of the deficit. Finally, a confirmatory diagnosis by a qualified professional, such as a paediatrician or educational psychologist, is required. Despite these problems, the DCDQ'07 was to be unbiased, with good reliability and validity (Wilson *et al.*, 2000) and some authors describe children to be "less-than-accurate reporters of their own behaviour" (Frick, Barry and Kamphaus, 2009). Furthermore, parent questionnaires can be a good measure of impact on daily living for the child; however, additional self-reporting questionnaires should be included (Klassen, Miller and Fine, 2006). Cut-offs for classification were also used.

This study used strict cut-offs defined by standard deviations to identify moderate and severe SPLDs. These cut-offs were based on standards set by the UK Joint Council for Qualifications (Joint Council for Qualifications, 2015) and therefore this data can be used to identify the number of patients that will potentially require educational support. Nevertheless, even with the cut-offs, there was a proportion of the control group who had lower scores. Controls fulfilling the criteria for an SPLD could mean that the control group contains individuals with deficits indicative of an SPLD or the cut-offs were too simplistic. This problem could be resolved by identifying if a deficit truly exists by using multiple tests and enquiring whether the participant has a real-life impairment. Furthermore, to rule out false positives the content scale of the CBRS could be used to see if the child had an impairment, for example, an indication for language or academic problems.

1.1.2 Measuring development delay

The statistical analysis of developmental milestones would have been improved if milestone data had been collected for the control group. Furthermore, the milestones were collected from the parents in the form of a questionnaire, and therefore the answers likely relied on the parent's memory rather than primary source material such as the child's personal child health record (PCHR). Despite these limitations, efforts were made to analyse the distribution of the data and perform stringent statistical testing. Graphical presentation of the milestone data revealed normal distributions, which would suggest a lack of bias in the data. Previously published data from large control datasets were obtained so an accurate comparison could be made. Finally, to reduce the number of false positives, strict cut-offs of 2.5 standard deviations from the control mean were used to identify potential delay. This conservative approach, however, does increase the risk of underestimating delay. These methodological approaches hopefully improved the analysis and interpretation of the milestone data.

1.1.3 Epilepsy-related confounds

All the cases had evidence of rolandic spikes and experience of several seizures during the study. Furthermore, around half of the individuals with RE were using drug therapies. It is quite likely that some of these features could influence cognition and the changes in cognition; however, this study did not try to analyse these confounds. The reasoning for this relates to retaining power for the study but also the findings within the literature. The side of spike and their frequency appear to produce mixed findings in relation to altered cognition (Wolff *et al.*, 2005; Bedoin *et al.*, 2006; Jurkevičienė *et al.*, 2012), this may be because the side and frequencies of spike appear to change over time (Ewen *et al.*, 2011; Vannest *et al.*, 2016). Similar problems were found in monitoring seizure frequency.

Seizure frequency is important to understand the severity of epilepsy. However, it is also easily misreported either due to poor identification of seizures (Akman *et al.*, 2009; Elger and Hoppe, 2018) or the reliance on memory to calculate the number of seizures before the baseline measures. Moreover, guidelines for which the seizure types to record would improve the data quality as different

seizure types recruit different brain regions, i.e. there could potentially be a difference in cognition between those with focal seizures only and those with additional generalised tonic-clonic seizures (Giordani *et al.*, 2006). The analysis of AED therapy also raises additional complications.

The effect of anti-epileptic drugs (AEDs) on cognition is an area of great debate in RE. This debate has been driven by many low powered studies which have produced results for and against the use effect of AEDs to improve cognition (Nicolai *et al.*, 2006; Piccinelli *et al.*, 2010). To be cautious, this study has avoided the debate as what is needed is high-quality data from a randomised control trial where the effects of one type of drug at a fixed dose are measured against no drug intervention. It is tempting to explore the relationship between the effect of AED therapy and cognition, but the extra data would do little to further the debate. This study has viewed the RE group as a homogenous group, which means that the results and their implications are broadly applicable to the entire patient group regardless of seizure severity or other epilepsy variables.

5.5.1 Change in assessor and environments between baseline and follow-up

An important point which may have influenced the collection of neuropsychological data was the change in assessors and environments between the baseline and follow-up. This is a valid criticism of the data however there is evidence to suggest that this factor may play a minimal role. Reasons for this interpretation include one, the neuropsychology was directed by Dr Anna Smith at both time points, at the follow-up time point the amount of testing was much reduced compared to the baseline and also at follow-up formal quiet environments were used to collect the data. For example the changes in testing at follow-up would be the ideal conditions for an improvement in the WASI matrices scores in the control group rather than a decline. A more likely factor for a decline in the control scores over time maybe that initial measurements had larger confidence intervals as children, and this interval has decreased as adolescents providing an improved measure of cognitive function in these participants. Another possibility is that many of the participants were assessed during a period of exam stress; this may have impacted their ability.

6 Changes in cortical thickness and subcortical volumes in children with Rolandic epilepsy between active epilepsy and seizure remission

6.1 Introduction

Rolandic epilepsy (RE) has normal clinical neuroimaging, yet there is evidence to suggest subtle differences in brain structure when compared to healthy children. Earlier in the thesis, the existing magnetic resonance (MR) neuro-imaging literature in RE was carefully analysed, and the following conclusions were made. The cross-sectional studies revealed evidence of both small regions of thicker and thinner cortex within and outside the central sulcus when compared to healthy controls. The thickness of altered cortex may have been influenced by the age of the participant; patches of thicker cortex were most apparent in younger children with RE compared to older children. The sole longitudinal study found evidence of reduced thinning of the cortex over two years compared to healthy controls(Garcia-Ramos *et al.*, 2015). Furthermore, in the control group, there was evidence of a diffuse reduction in cortical thickness with time which is in keeping with recent studies of cortical development in healthy children (L. M. Wierenga *et al.*, 2014). These findings would suggest a possible delay in the neurodevelopment of the cortex in individuals with RE. Evidence of a delay is also apparent in the volumes of subcortical structures.

The earlier chapter also identified that an enlarged putamen was a consistent finding in studies of subcortical volume in children with RE when compared to healthy controls. This apparent enlargement had a large effect size. Furthermore, the sole longitudinal study found that the putamen slightly increased in volume over time in those with RE, whereas in healthy controls, its size was seen to decrease. The developmental difference would suggest that enlarged putamen volume is a discrete morphometric feature of the brains of children with RE which is inherent to both active epilepsy and seizure remission. Further research may reveal it to be a biomarker; however, for this to be the case, there is a need to understand better how grey matter thickness and volume are measured using magnetic resonance imaging.

Magnetic resonance imaging of the brain is a quick and safe way of producing detailed images of brain structure (Lenroot and Giedd, 2006). Furthermore, due to the absence of ionising radiation, it can be repeated frequently with no adverse effects which makes it ideal for monitoring the development of a child's brain (Mills and Tamnes, 2014). Below is a brief overview of the generation of the MR signal.

The MR signal is a product of the averaged resonance of mainly free protons, hydrogen atoms without electrons, within a static magnetic field. As the proton has no electrons, the positive atomic nucleus generates a magnetic (dipole) moment. When placed in a static magnetic field (B_0), most of the magnetic moments align in orientation with this field. Even though the moments align, they are not stationary, which leads to the precession of the protons at a frequency known as the Larmor frequency. This precession of the protons magnetic moment is known as a spin, with the use of additional electromagnetic fields the spin can be manipulated to produce a signal.

Two extra magnetic fields are used to evoke a signal from the spin; the first generates a magnetic gradient for spatial location and the second is an oscillating magnetic field of radio-frequency pulses set to the resonant frequency of the spin. The recording of the signal generated by the spins is re-assembled into an image which closely reassembles the tissue of interest. There are different ways to measure the resulting signal and this results in different contrasted images. In this study, a T1 signal, otherwise known as T1 weighted relaxation, was used.

Generation of a T1 signal can be done by using a spin-echo (SE) or gradient-echo (GE) sequence. In this study, a GE sequence was used as it allowed for a shorter time to repeat (TR) and time to echo (TE) which is ideal for rapid acquisition of brain images in un-sedated children who are susceptible to moving. However, a limitation of this technique is a lower signal to noise ratio due to the refocusing of spins that have been dephased by the magnetic gradient. The T1 signal reflects the time taken for the spin to return to a resting spin orientation within the main static magnetic field. T1 relaxation produces high signal intensity in the white matter and lower signal intensity in the grey matter resulting in an

Figure 31: Axial T1-weighted MRI scan of a male aged 12 years.There is varying contrast in recorded MR signal due to the duration of decay in the T1 signal. Dark areas have a long decay and lighter areas have a short decay.

image similar to the one shown in Figure 31. The reason for this distribution of intensity is due to the variation in T1 relaxation in tissue. Free water and cortex take have longer T1 relaxation compared to myelinated white matter, which is short.

As mentioned, the recorded signals are a representation of the structure of the brain, which matters in the interpretation and analysis of the images. Biologically, the division between grey and white matter is not as clear as what is represented by an MR image. The grey matter consists of more than just cortical neurons. The measure of grey matter is composed of two-thirds cortical tissue. The remainder consists of glial cells, capillaries and a relatively large amount of extracellular space (Figure 32). The composition of grey matter has importance in understanding MR measurements of the cortex. As a result, modification of the MR signal can be induced by changes in any one of these components. For instance, the pruning of dendrites, changes in glial density or both could influence the apparent thickness of the grey matter in the developing child (Bennett and Lagopoulos, 2015).

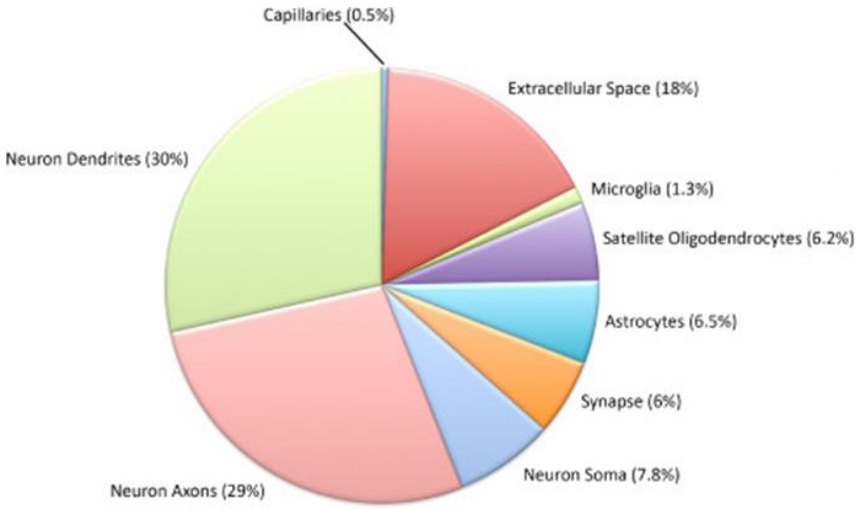


Figure 32: Neural and non-neural components within the cortical grey matter. Around two-thirds of the cortical grey matter consists of neuronal cell, neuronal projections or synapses. Extract from Bennet et al (2015).

6.2 Hypotheses

This longitudinal study will be the first to explore how brain structure changes in seizure remission in children with RE. The objectives are to recreate the Garcia-Ramos study (Garcia-Ramos *et al.*, 2015) to investigate changes in cortical thickness across the whole cortex and changes in the putamen, caudate and thalamus between active epilepsy and seizure remission. In addition, three *a priori* hypotheses will be tested. These relate to cortical thickness in the cortical regions involved in speech, language and reading and are based on the reviews conducted earlier in this thesis.

One, there will be a delay in the thinning of the cortex over regions connected by the auditory to the motor stream which processes speech sounds, converts to language and then facilitates the production of speech sounds. The regions in this stream include the posterior temporal cortex (superior temporal gyrus and transverse temporal gyrus), inferior parietal (i.e. angular gyrus and supramarginal gyrus) and inferior frontal regions (pars triangularis and pars opercularis) within the left hemisphere. These regions were chosen as children with RE can have problems with sub-lexical skills such as an inability to convert graphemes to phonemes (Smith, 2015), phonological working memory (Northcott *et al.*, 2007) and articulation and naming problems (Lundberg *et al.*, 2005) which are all features of dyslexia (Peterson and Pennington, 2015). Also, the same regions with be explored in the right hemisphere as they may have structurally compensated for developmental problems in the left hemisphere (Xing *et al.*, 2016).

Two, the putamen, caudate and thalamus in children with RE will be larger in volume compared to healthy controls. In particular, the putamen is bilaterally enlarged in four separate studies (Lin *et al.*, 2012; Cheng Luo *et al.*, 2015; Garcia-Ramos *et al.*, 2015; Kim *et al.*, 2015) making it a prime putative neuroimaging biomarker for RE. Furthermore, at follow-up, there will be a difference in the change in the volume of the putamen between the two groups, which leads to the final hypothesis.

Third, the putamen over time will remain enlarged compared to controls in the follow-up measurements. Moreover, between the two time-points in the RE group, there will be a small increase in volume in the left putamen and a greater increase in volume in the right putamen compared to the left. Whereas, in the control group, bilateral putamen volume will decrease, and this will be greatest

within the right putamen compared to the left. These hypotheses are based upon the longitudinal findings of Garcia-Ramos (Garcia-Ramos *et al.*, 2015).

The measurement of these features will help with the understanding of the differences in brain structure between children with RE and healthy controls at baseline and in seizure remission. It may help identify regions involved in the generation of seizures which could be used as biomarkers to improve the ability to diagnose patients with this form of epilepsy. Furthermore, it may be able to identify possible reasons for co-occurring cognitive problems in children with RE.

6.3 Methods

6.3.1 Participants

Recruitment of participants for the longitudinal study began in 2012. Thirty-one children with RE were recruited from hospitals in South England. All diagnoses had to fulfil the ILAE criteria for the classification of the epilepsies (Engel, 2001) and Rolandic spikes (RS) had to be apparent on EEG. The participants had to have more than one seizure, and the seizures had to be focal in semiology with a clear sensory and motor component. Participants with epilepsy were still included if they had experienced status epilepticus. Participants with co-occurring cognitive issues were included in this study, as long as there was no evidence of developmental regression. Exclusion criteria included abnormal MRI features such as hippocampal sclerosis, space-occupying lesions, white matter lesions, and malformations of cortical development (Duncan, 1997). Individuals with other neurological problems were excluded except for those with migraine and anisocoria

At follow-up, the participants were reviewed, seven of these children (22.6%), did not fulfil the diagnostic criteria for RE and were subsequently removed from the study. In three female participants, they all had a deterioration of seizures and were either classified as having an evolution of RE or were re-diagnosed. One had refractory temporal lobe epilepsy and was on a surgical pathway, another had developed absences with photosensitivity, and the third had intractable seizures and diagnostic uncertainty. A further four males had diagnostic uncertainty. The first, despite a normal MRI at baseline, had atypical seizures which presented with bilateral paraesthesia in the face with blurred vision. At baseline and follow-up, he also had continuous high amplitude slowing of the EEG over the right posterior temporal and occipital regions. The second, appeared to have been misdiagnosed with RE due to unexplained nocturnal events with intense sweating and anxious behaviour which developed into a semi-responsive state with “glazed eyes”. He had RS on EEG, but upon review, it was decided that this participant did not fulfil the strict criteria for the study. The third had at the onset a semiology similar to Rolandic seizures but developed additional absences and poor seizure control. The final male had only one seizure during the study period, and their EEG was negative for RS and thus did not fulfil the inclusion criteria.

Twenty-five healthy controls were recruited from the community by advertisement. These participants had to be fit and healthy and have a history absent from neurological problems. At baseline, they had to score above 80 in both the verbal and performance components of the WASI II intelligence quotient test (Wechsler and Zhou, 2011). Furthermore, there had to be a negative family history of neurological problems or neurodevelopment problems. At follow-up, a review of history was performed on all participants; one male participant had a first-degree relative who had developed idiopathic generalised epilepsy and another participant, who scored below 80 in verbal IQ at baseline, were removed from the study.

After removal of participants at follow-up, there were 24 adolescents with RE in seizure remission and 23 healthy controls were eligible for follow-up MRI. In the RE group, ten participants could not be scanned: seven did not return, two had fixed orthodontic braces, which would have marred the MR image, and one refused to be scanned. In the control group, six could not be scanned: four did not respond, one had suspected metal in his eye, and one had a fixed orthodontic brace.

The collection of follow-up scans consisted of 12 from both the RE and control scans. However, a visual quality check of the scans resulted in the rejection of two scans from the RE group as there were visible signs of motion artefact.

6.3.2 MRI data acquisition

MRI data were collected from 2012 to 2018. T1-weighted 3D volumetric images were acquired sagittally on a 3-Tesla Signa HDx scanner (General Electric Medical Systems, Milwaukee, USA) at the Centre for Neuroimaging Sciences (Institute of Psychiatry, Psychology & Neuroscience, King's College London, UK). The scanner utilised an Alzheimer's Disease Neuroimaging Initiative (ADNI) magnetisation-prepared 180 degrees radio-frequency pulses and rapid gradient-echo (MPRAGE) acquisition protocol. The following parameters were used: time to echo (TE) = 2.848 ms, repetition time (TR) = 6.988 ms, inversion time = 650ms; flip angle = 8 degrees; slice thickness = 1.2 millimetres (mm), spatial positions = 166; matrix = 256 x 256 and field of view = 260. The participants head was kept in a fixed position by using pillows.

The follow-up scans occurred 4.58 years after the baseline. Due to the decommissioning of the baseline scanner, a different scanner was used at follow-up. The replacement scanner was a GE MR750 GE 3-Tesla and was situated within the Centre for Neuroimaging Science. Similar parameters were used for the acquisition of the MR data. Time to echo (TE) = 3.024 ms, repetition time (TR) = 7.328 ms, inversion time = 400 ms; flip angle = 11 degrees; slice thickness = 1.2 millimetres (mm), spatial positions = 196; matrix = 256 x 256 and field of view = 2 70. These parameters were designed to closely replicate the legacy ADNI MPRAGE scan protocol used at baseline.

6.3.3 Image processing and measurements of cortical thickness

MR images were processed using FreeSurfer (version 6.0), which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/) (Fischl, 2012). T1-weighted MR images were used for cortical reconstruction and volumetric segmentation. This process includes motion correction and averaging (Reuter, Rosas and Fischl, 2010) of multiple volumetric T1 weighted images and removal of non-brain tissue. This utilises a hybrid watershed/surface deformation procedure (Ségonne *et al.*, 2004), automated Talairach transformation, segmentation of the subcortical white and grey matter volumetric structures (Fischl *et al.*, 2002, 2004), intensity normalization (Sled, Zijdenbos and

Evans, 1998), tessellation of the grey/white matter boundary, automated topology correction (Fischl, Liu and Dale, 2001; Segonne, Pacheco and Fischl, 2007) and surface deformation following the MR image intensity gradients to optimally place the grey/white and grey/cerebrospinal fluid borders. The delineation of this border is where there is the greatest shift in intensity; this relies on the assumption that the shift represents the transition to another other tissue class (Fischl and Dale, 2000). Once the cortical models are complete, several deformable procedures can be performed for data processing. These deformable procedures, include surface inflation (Dale, Fischl and Sereno, 1999), registration to a spherical atlas, which is based on the participants unique cortical folding patterns to allow for the comparison of cortical geometry between participants (Fischl *et al.*, 1999), and parcellation of the cerebral cortex into units with respect to gyral and sulcal structure (Fischl *et al.*, 2004; Desikan *et al.*, 2006). A visual overview of these processes is presented in Figure 33. This method uses both intensity and continuity information from the entire three dimensional MR volume in segmentation and deformation procedures to produce surfaces which can be used to calculate cortical thickness (Fischl and Dale, 2000).

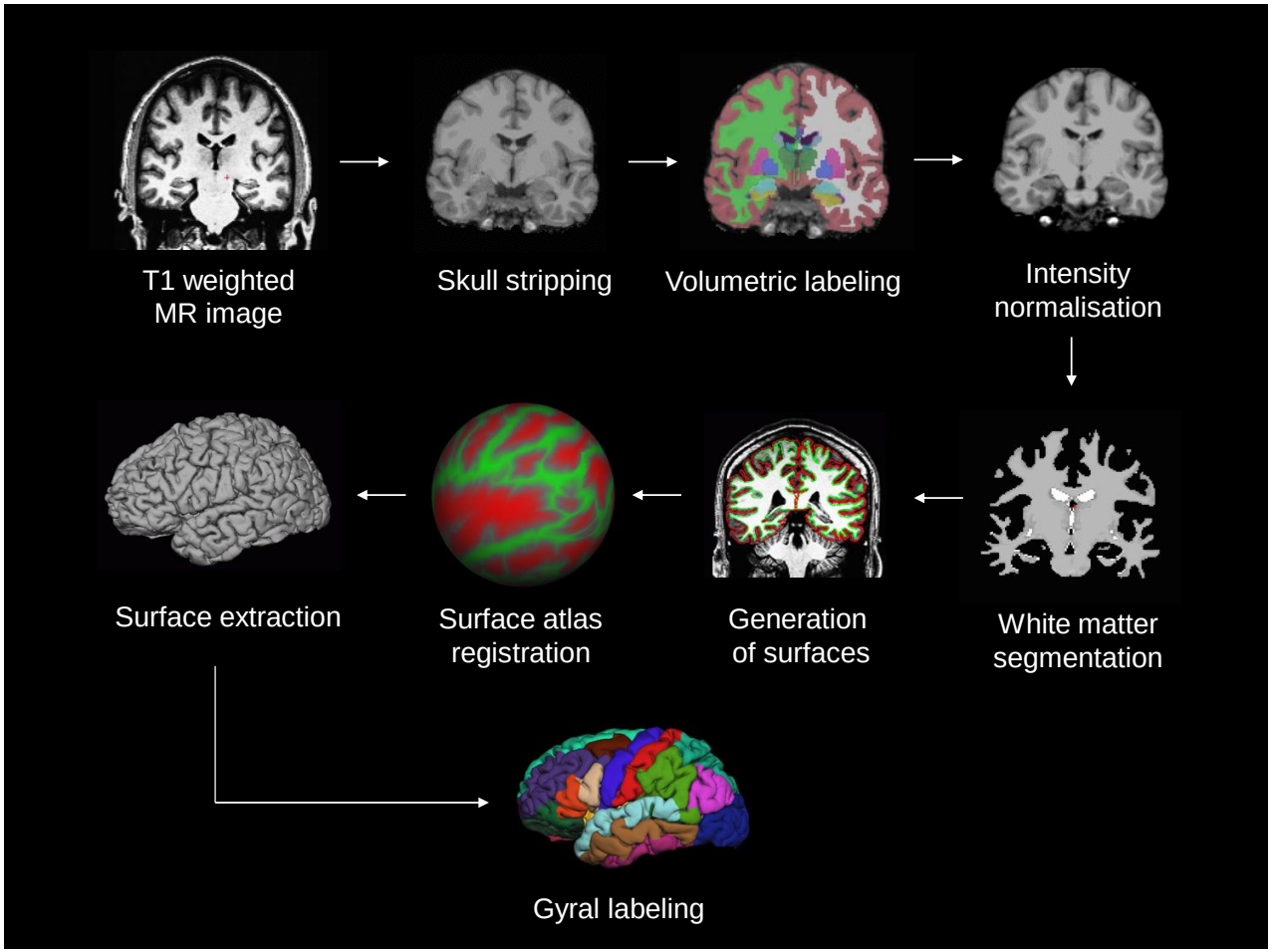


Figure 33: FreeSurfer pipeline from MRI to 3D brain model. This figure is a simplified interpretation of the FreeSurfer computational process in this study. The input was a T1 weighted MR brain image. The skull and any none brain features are stripped away. The resulting brain volume is then labelled into different tissue types which include grey and white matter and a variety of subcortical structures. Intensity normalisation is used to transform the white and grey matter into a uniform intensity thus allowing a clear delineation between the two tissue types. White matter segmentation involves defining the white matter space. It is important to note that subcortical grey matter structures and the ventricles are filled in in this process creating a solid volume. The next step produces a transformation from the volume space to a surface space. Surfaces are generated across both the border of white matter and grey and the border between grey matter and the pial membrane. The generation of the white matter surface is more complex than white matter volumetric segmentation as it involves following T1 intensity gradients and there is a smoothness constraint. The generation of the grey matter surface relies of the white matter surface as a starting point and move out from this surface following the intensity gradient till there drop indicating the edge of the grey matter. The resulting surfaces are then inflated into a sphere which allows for sulcal and gyral alignment with a template. The surface is then put back into the original space to produce a 3D surface. Gyral labelling using the Desikan-Killany atlas is initiated during the surface atlas registration however it is fine tuned to the individual's anatomy post surface extraction. Images and description extracted and adapted from http://surfer.nmr.mgh.harvard.edu/fswiki/Tutorials

Cortical thickness values were then calculated. The measure of cortical thickness is the average of the closest distance from the grey/white matter boundary to the grey/pial boundary at each vertex on the surface and the distance from the grey/pial boundary to the grey/white matter boundary at each vertex (Fischl and Dale, 2000). Each hemisphere of a brain reconstruction is created by a mesh surface using fast triangle-triangle intersections (Fischl, 2012). Each hemisphere mesh consists of around 140,000 triangles, a collection of triangles which forms an intersection is known as vertices. Across the mesh, the distance between vertices is around 1 mm.

Freesurfer is a sophisticated tool for measuring cortical thickness as the generated maps of cortical thickness are created using spatial intensity gradients across tissue classes and are therefore not simply reliant on absolute signal intensity. Moreover, the measurements are not restricted to the voxel resolution of the original data resulting in the detection of sub-millimetre differences in cortical thickness between groups. (Fischl and Dale, 2000) Furthermore, cortical folding complicates the measurement of cortical volume because the folds do not align with the cardinal x, y and z-axes used in viewing slice data. As a result, data extracted from MRI volumes rather than surfaces result in a lack of accuracy and over-estimations (Fischl and Dale, 2000).

FreeSurfer was used in this study as it has many features which make it superior to other neuroimaging analysis tools. The procedure for the measurement of cortical thickness has been validated against histological analysis (Rosas *et al.*, 2002) and manual measurements (Salat *et al.*, 2004). Furthermore, FreeSurfers morphometric procedures have been demonstrated to show good test-retest reliability across scanner manufacturers and MRI field strengths (Han *et al.*, 2006; Reuter *et al.*, 2012).

An additional quality check was performed on the reconstructed brain surfaces. The check was done using the internal quality check method contained within the ENIGMA Cortical Quality Check 2.0 (http://enigma.ini.usc.edu/). This method checks for cortical segmentation quality and is good for identifying under and over-estimations. Also, an external visual check was performed on all recons. Subcortical volumes were checked using ENIGMA 3 – GWAS meta-analysis of subcortical volumes (http://enigma.ini.usc.edu/). These stringent techniques were used to make sure that the neuroimaging data were of the best quality comparable to data analysed by the ENIGMA-Epilepsy consortium (Whelan *et al.*, 2018).

Finally, the data were automatically processed using the longitudinal stream of the Freesurfer (6.0) (Reuter *et al.*, 2012). Using robust, inverse consistent registration an unbiased within-participant template, a volume is produced (Reuter et al., 2010). This new volume is unbiased with respect to any time-point and is known as a “base”. The base is then used to perform processes which include skull stripping, Talairach transforms, atlas registration, the generation of spherical surface maps and cortical parcellation in each of scan within participants. This technique reduces random variation in the processing procedure and improves the robustness, sensitivity and reliability of the overall longitudinal analysis and output data.

6.3.4 **Statistical analysis of cortical thickness and subcortical volumes**

6.3.4.1 *Cortical thickness surface analysis*

Measurements of longitudinal changes in cortical thickness and subcortical volumes were created by converting the change into a rate and symmetrised per cent change (SPC). Rate creates a value of mm/year, and SPC is a percentage of change, which considers the mean cortical thickness between the two time-points. SPC is the recommended and the most robust method for measuring change; however, it is an abstract figure. Hence the rate of change was also calculated.

$$rate\ of\ change = \frac{(cortical\ thickness\ TP2 - cortical\ thickness\ TP1)}{(age\ TP2 - age\ TP1)}$$

Equation 1: Rate of change in cortical thickness (mm/year).Timepoint 1 (TP1) is the baseline, and time point 2(TP2) is the follow-up.

$$SPC = 100 * \frac{rate\ of\ change}{average\ cortical\ thickness}$$

Equation 2: Symmetrised per cent change in cortical thickness.Rate of change is calculated from Equation 1. The average measurement is the measure from TP1 and TP2 divided by two. To convert into a percentage, the result is multiplied by 100.

SPC is a more robust than per cent change because thickness at time-point one is noisier than the average, plus the output of SPC is symmetric, for example, when reversing the order of time-point 1 and time-point 2, SPC has a change from positive to negative or vice-versa which is not true for per cent change (https://surfer.nmr.mgh.harvard.edu/fswiki/LongitudinalTwoStageModel).

When the rate of change and SPC are compared between groups, their values can be widely different, even reversed. To explain why this is the case, the figure below will demonstrate how regions with the same rate of change in cortical thickness could have different values of SPC.

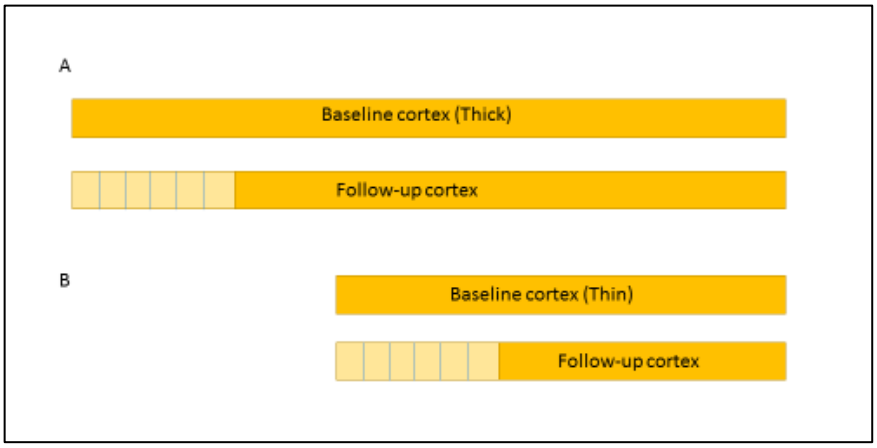


Figure 34: The difference between rate of change in cortical thickness and measurements of SPC. In dark yellow are the cortical measurements and in light yellow are the incremental average changes in cortical thickness. These changes are identical between A and B and thus the same rate measurement would be obtained. SPC differs as it divides the rate by the average cortical thickness, therefore as thickness is large in A at baseline and at follow-up the value of SPC will be larger whereas in thinner cortex at baseline and at follow-up the average will be smaller and will thus result in a larger value for SPC.

The measures of the rate of change and SPC were analysed in two ways. A simple analysis of mean SPC per cortical parcellation in the Desikan-Killany atlas and sophisticated analysis of every vertex across each hemisphere. Using SPSS, a simple analysis was performed between the average values at each time-point using a multiple analysis of covariance (MANCOVA) test with sex and age as covariates. Levene's test for equality of variances was used to assess whether data in a group had a larger variance which would affect the accuracy of the t-test.

Vertices based cluster statistics were also used to measures differences in cortical thickness. Cluster analysis was done using FreeSurfers statistical tool for vertices-based analysis called Qdec. The data was smoothed using 15 full-width at half-maximum (FWHM) kernel, and differences in SPC were analysed corrected for age and sex. A significant cluster had to fulfil two criteria; one, the intensity of the cluster must fall within the intensity threshold criteria and two, it must be part of a contiguous set of vertices that meet the threshold criteria. There was no restriction on the minimum surface area (https://surfer.nmr.mgh.harvard.edu/fswiki/mri_surfcluster). All significant clusters were corrected for multiple comparisons.

The output was corrected for multiple comparisons using a Qdec Monte-Carlo null Z simulation. The simulation synthesises a Z map and to reduce inter-subject variability; the data residuals were

smoothed with a 15 mm FWHM kernel. The z map is then thresholded to p=<0.05 in either a positive or a negative direction. The simulation then finds clusters on the map and records the area of the maximum cluster. The simulation then repeated this process over a desired number of iterations (usually >5000) (https://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/GroupAnalysis#ClusterwiseCorrectionforMultipleComparisons). This involves going back to the original data and thresholding using the same p-value and direction of change. The simulation then finds clusters in the thresholded map and for each cluster p= probability of seeing a maximum cluster that size or larger during the simulation. This sophisticated analysis of SPC is best understood when combined with actual measures of cortical thickness.

The final, analysis of cortical data involved measures of thickness at baseline and follow-up. The analysis was performed in two ways, an average of cortical thickness for each hemisphere and regional analysis of Desikan-Killany parcellated regions, which was part hypothesis lead and part exploratory. To avoid bias, the exploratory analysis did not include hypothesised regions from the analysis. All statistical comparisons were made using multiple analysis of variance (MANOVA) with age and sex as covariates unless otherwise specified.

6.3.4.2 *Subcortical volume analysis*

Subcortical volumes were also extracted; this was restricted to the hypothesised putamen, caudate and thalamus. Using OASIS-3 guidelines, these volumes were corrected for intracranial volume (version 1.5, March 2018). Correcting ICV involved calculating the mean ICV for the control group and then calculating a regression with ICV as an independent variable and ROI as a dependent variable to obtain the B weight (Tsai *et al.*, 2013). The B weight was then used to calculate normalised volume using the following Equation 3.

$$Normalised\ volume = raw\ volume - (B\ weight * (sp\ ICV - mean\ ICV))$$

Equation 3: Normalised volume for individual subcortical structures. The raw volume is that which is measured by Freesurfer segmentation. The B weight is calculated from the regression of ICV (independent variable) against the volume of the structure of interest. Mean ICV of the control group is subtracted from the ICV of a single participant (sp ICV). This value is multiplied by the B weight and then subtracted from the raw volume.

OASIS-3 guidelines do not recommend correcting cortical thickness measurements as there is little relationship between ICV and cortical thickness, this is because volume scales with head size, which is mostly due to changes in surface area, whereas thickness alone scales to a much less degree (Schwarz *et al.*, 2016). The subcortical structure had their volumes compared between groups in a cross-sectional design. Mean control ICV was calculated for each time-point resulting in a difference in values used in Equation 3. In addition, longitudinal measurements of the rate of change (Equation 1) and SPC (Equation 2) were calculated between baseline and follow-up with cortical thickness replaced with volume in the equation. All volumes in the longitudinal analysis were corrected using Equation 3. The cross-sectional and longitudinal results between groups were compared in a MANOVA with sex and age as covariates.

6.4 Results

6.4.1 Demographics

The number of scans per group at each time-point with demographic information are presented below (Table 39). The average amount of time between scans was 4.14 ± 0.81 years in the RE group and 4.97 ± 0.26 in the control group, this was not significant (p= 0.079). In the RE group, at baseline, as reported from their earliest EEG, twelve had right-sided, or right dominant spikes and seven had left side or dominant spikes. At follow-up, eight had a right side or right dominant spikes, and two had left side or left dominant spikes. The incidence of status epilepticus (SE) is reported if the participant experienced SE at any time during the study. At time point one, this was four individuals, and at time point two, two individuals. Their parents reported seizures, which lasted between 10 minutes to 2.5 hours. All of these participants experienced SE only once.

Table 2 contains the demographics of those individuals who had a scan at both the baseline and follow-up and were of suitable quality to be included in a longitudinal analysis. The average amount of time between the scans in the control group was 5.16±0.76 years and 4.32±0.63 in the RE group, and this was found to be significant (p=0.018). In the longitudinal group, seven participants with RE had a right side or right dominant spikes, and one had left side or left dominant spikes. Incidence of SE in the longitudinal study was 25% with the longest seizure lasting for 2.5 hours.

Group/time-point (number of scans)	RE TP1 (19)	Con TP1 (22)	DF	Stats (p=)	RE TP2 (10)	Con TP2 (12)	DF	Stats (p=)
Age (years±sd)	10.9±1.68	12.10±1.69	40	**0.026**	14.63±2.4	17.69±2.29	21	**0.007**
Sex (% male)	68.4	54.5	40	0.36	70	50	21	0.34
Handedness (% right-handed)	94.7	100	40	0.88	90	100	21	0.85
AEDs (%)	57.9	N/A		N/A	50	N/A		N/A
Age of onset (years)	7.64±1.78	N/A		N/A	6.59±2.44	N/A		N/A
Duration of epilepsy (months)	38.88±23.38	N/A		N/A	60.6±30.10	N/A		N/A
Evidence of status epilepticus (%)	21				20			
Age at final seizure (years)	N/A	N/A		N/A	11.63±2.15	N/A		N/A
Duration of seizure remission					35.96±21.59			

Table 39: Demographics of participants with RE and healthy controls who had cross-sectional MR imaging. Stats: Statistical comparison of age by t-test, sex and handedness by χ^2. DF: Degrees of freedom Evidence of status epilepticus was reported if it had occurred at any time during the study. There was a significant difference in age between the groups at both baseline and follow-up.

Group (number of scans)	RE (8)	Con (12)	DF	Stats (p=)
Age TP1 (years±sd)	10.9±1.91	12.5±1.87	19	0.077
Age TP2 (years±sd)	15.23±2.3	17.69±2.3	19	0.033
Sex (% male)	62.5	50	19	0.582
Handedness (% right-handed)	87.5	100	19	0.209
AEDs baseline (%)	4 (50%)			
AEDs follow-up (%)	4 (50%)			
Age of onset (years)	7.02±2.23			
Duration of epilepsy (months)	59.9±27.8			
Evidence of status epilepticus (%)	2 (25%)			
Age at seizure remission (years)	12.01±2.23			
Duration of seizure remission (months)	38.53±23.7			

Table 40: Demographics of participants with RE and healthy controls who had longitudinal MR imaging. Stats: Statistical comparison of age by t-test, sex and handedness by χ^2. DF: Degrees of freedom. Evidence of status epilepticus was reported if experienced at any time during the study. There was a significant difference in age between the two groups at follow-up.

6.4.2 **Cross-sectional measures of cortical thickness**

6.4.2.1 *Interhemispheric comparison*

The average cortical thickness (mm) was compared between the groups at different time-points. The first analysis was hypothesis led; it was hypothesised that there would be thicker cortex in regions that are believed to be related to cognitive problems in RE. The regions included; in the frontal lobe; pars triangularis and opercularis, in the temporal lobe; superior temporal gyrus and the transverse temporal gyrus and the parietal lobe the supramarginal gyrus and inferior parietal regions. In addition, to these hypotheses, a global exploratory analysis of all cortical regions was performed. Box plots were created for the average cortical thickness in the left and right hemisphere at baseline and follow-up, to analyse the distribution of the data and identify outliers. (Figure 35)

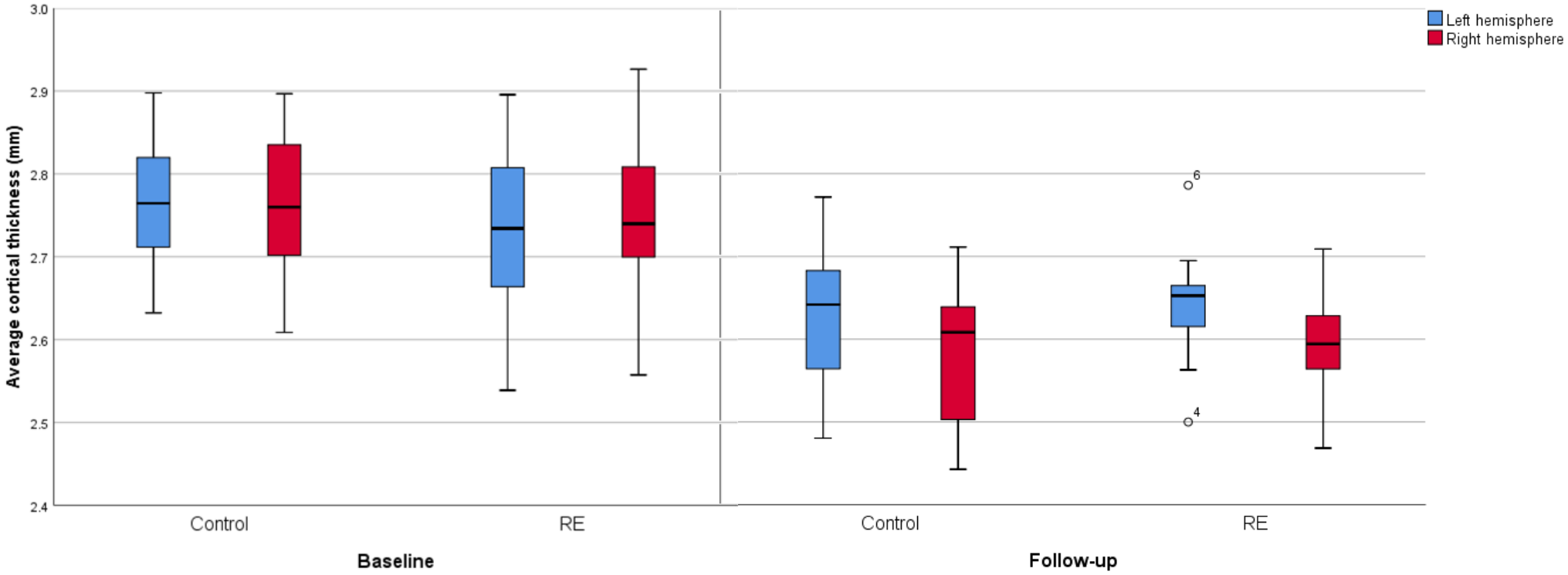


Figure 35: Boxplots of cortical thickness between participants with RE and healthy controls at baseline and follow-up. Average cortical thickness of left hemisphere (blue) and right hemisphere (red). Y axis range from 2.4 to 3 mm. The same scale of average cortical thickness is used in each panel. Baseline (left panel) 22 controls and 19 individuals with RE and follow-up 12 controls and 10 individuals with RE (right panel). In the baseline MANOVA analysis with age and sex as covariates found no statistical difference in the average cortical thickness of the left hemisphere (p=0.744) and no statistical difference in the right (p=0.962). Similarly, in the follow-up there was no significant difference in the left (p=0.735) or right (0.914) hemispheres.

At baseline and follow-up, there was no statistical difference in the average cortical thickness of the left and right hemisphere between the two groups. MANOVA analysis revealed no significant difference between the groups and no effects were introduced by the covariates. Despite this at baseline, the average cortical thickness was marginally larger in controls compared to those children with RE. Furthermore, the RE group showed a greater variance in thickness compared to healthy controls. At follow-up, the average cortical thickness in the control group was thinner than the RE group. Contrary to the baseline, the variance was reduced in the RE group and remained similar in the control group. Between the hemispheres, in both groups, the left hemisphere was thicker at follow-up, and this difference in thickness was to a similar extent. At follow-up, Figure 35 reveals outliers in the data set in the left hemisphere of the RE group, as the outliers were only within the left hemisphere and there had been a reduction in the sample size at follow-up, a decision was made to keep these data in the analysis.

6.4.2.2 *Left hemisphere*

6.4.2.2.1 Hypothesised regions

There was no significant difference between the hypothesised regions at baseline or at follow-up. A MANOVA model with the covariates sex and age found no significant difference between the groups at baseline ($p = 0.803$) and follow-up ($p = 0.986$). The average cortical thickness of hypothesised regions and baseline and follow-up are presented in Table 41: Left hemisphere average cortical thickness for hypothesised regions in controls and participants with RE at baseline and follow-up. At baseline, mean cortical thickness for the hypothesised regions except for the pars triangularis in controls was larger in the RE group. In the follow-up cohorts, a reverse picture was seen, the controls had greater cortical thickness compared to the RE group in all of the regions except for the inferior parietal regions.

	Baseline				Follow-up			
	Control n=22	RE n=19	DF	Stats	Control n = 12	RE = 10	DF	Stats
Pars Triangularis (mm)	2.74 ± 0.14	2.74 ± 0.22	38	0.995	2.52 ± 0.17	2.51 ± 0.11	19	0.887
Pars Opercularis (mm)	2.85 ± 0.12	2.81 ± 0.20	38	0.838	2.70 ± 0.18	2.66 ± 0.19	19	0.383
Superior temporal gyrus (mm)	3.05 ± 0.16	3.00 ± 0.12	38	0.183	3.03 ± 0.12	2.98 ± 0.17	19	0.670
Transverse temporal gyrus (mm)	2.73 ± 0.18	2.61 ± 0.23	38	0.300	2.65 ± 0.18	2.58 ± 0.27	19	0.642
Supra-marginal gyrus (mm)	2.84 ± 0.13	2.80 ± 0.20	38	0.777	2.76 ± 0.08	2.73 ± 0.15	19	0.925
Inferior parietal region (mm)	2.79 ± 0.08	2.77 ± 0.15	38	0.694	2.62 ± 0.10	2.65 ± 0.10	19	0.635

Table 41: Left hemisphere average cortical thickness for hypothesised regions in controls and participants with RE at baseline and follow-up. Cortical regions defined from the Desikan-Killany atlas. Statistics calculated by MANOVA with covariates age and sex. DF: Degrees of freedom There was no significant difference between the hypothesised regions in the two groups at baseline or at follow-up.

6.4.2.2.2 Exploratory analysis

	Follow-up				
	Control n = 12	RE = 10	DF	Stats	Effect size (d)
Rostral middle frontal region	2.33 ± 0.11	2.37 ± 0.14	19	**0.002** [a]	0.31

*Table 42: Exploratory analysis within the left hemisphere. Presented are regions significant or close to significance. All regions are defined from the Desikan-Killany atlas. Values are in millimetres. The statistical analysis used MANOVA with age and sex as covariates. DF: Degrees of freedom **Bold** stats are significant. [a] did not survive Bonferroni correction. Reported effect size Cohens d.*

Exploratory analysis found no regions of difference at baseline and one region of difference at follow-up (Table 42). In the follow-up, the rostral middle frontal region was the only significant area; no other regions approached significance. The rostral middle frontal regions were thicker in children with RE compared to healthy controls. The rostral middle frontal region is a large area within the frontal lobe which spans between the posterior, caudal middle frontal region and the anterior, lateral orbitofrontal region. Lateral and inferior to these regions were the hypothesised regions of the pars triangularis and opercularis. Despite the significant difference, the effect size was small, which could mean that this data could be false positive.

6.4.2.2.3 Cortical thickness maps

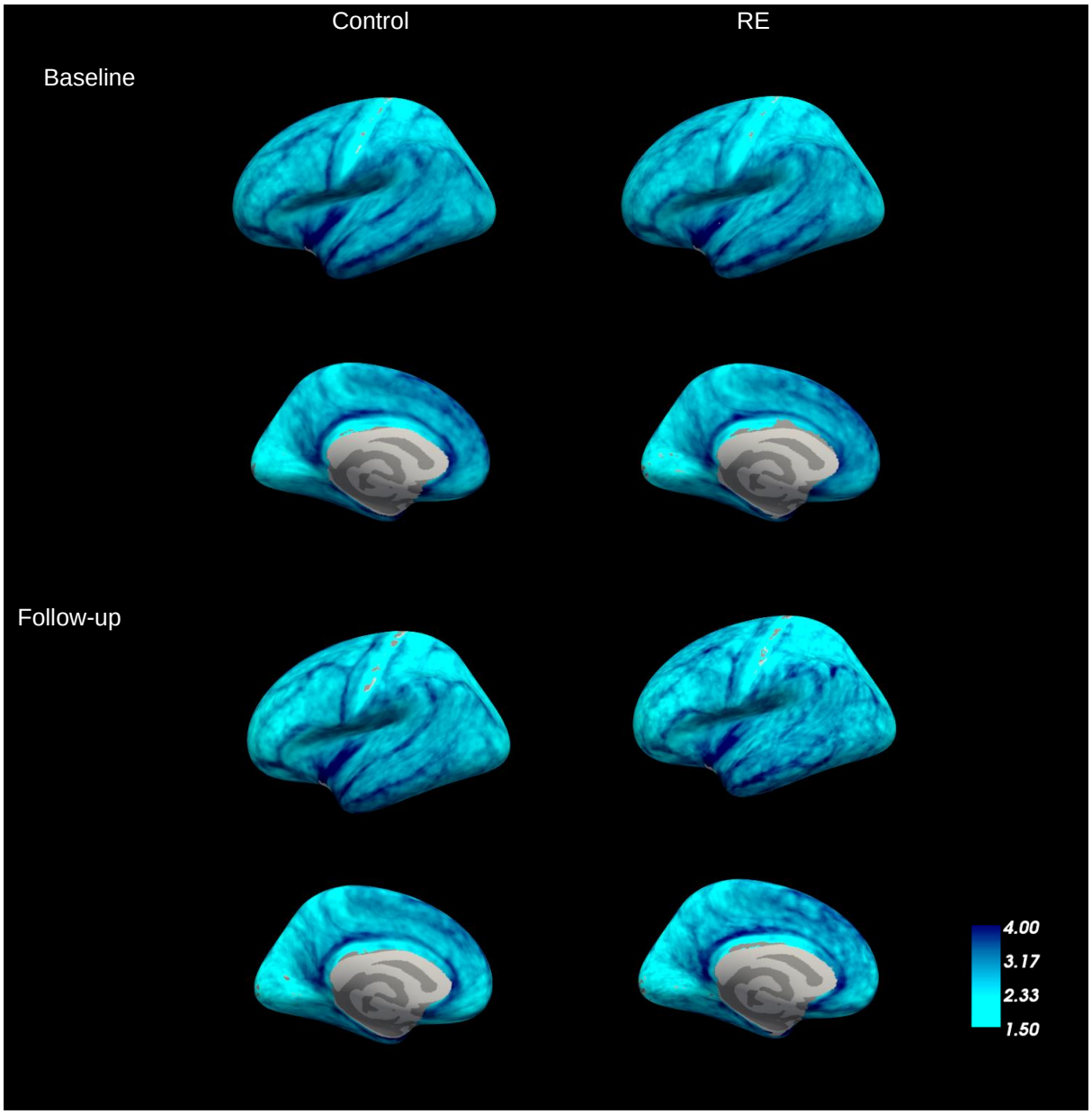


Figure 36: Cortical thickness maps of the left hemisphere in a composite of participants with RE and healthy controls. Cortical thickness is displayed by a blue overlay over an inflated pial surface. Cortical thickness measures range from 1.5 mm (light blue) to 4 mm (dark blue). Any regions of the cortex which are less than 1.5 are in grey. A brief assessment of these images reveals little difference; however, when you look in more detail there are subtle differences in cortical thickness.

In Figure 36, vertices maps of cortical thickness for the left hemisphere at baseline and follow-up are presented. Subtle differences between the groups can be seen at baseline between the two groups. In the RE group, gyral ridges are not as well defined. Furthermore, the extent of the thicker cortex in the inferior frontal gyrus appears to be reduced. Patches of cortex thinner than 1.5 mm are more

prevalent in the central sulcus and less prevalent along the cingulate gyrus and lateral occipital cortex in controls compared to the RE group. A similar picture of thin cortical regions and ill-defined gyral ridges is seen at follow-up. Furthermore, in the RE group, the hemispheres are darker and mottled colour compared to controls indicating a thicker cortex.

6.4.2.2.4 SPC vertices-based analysis

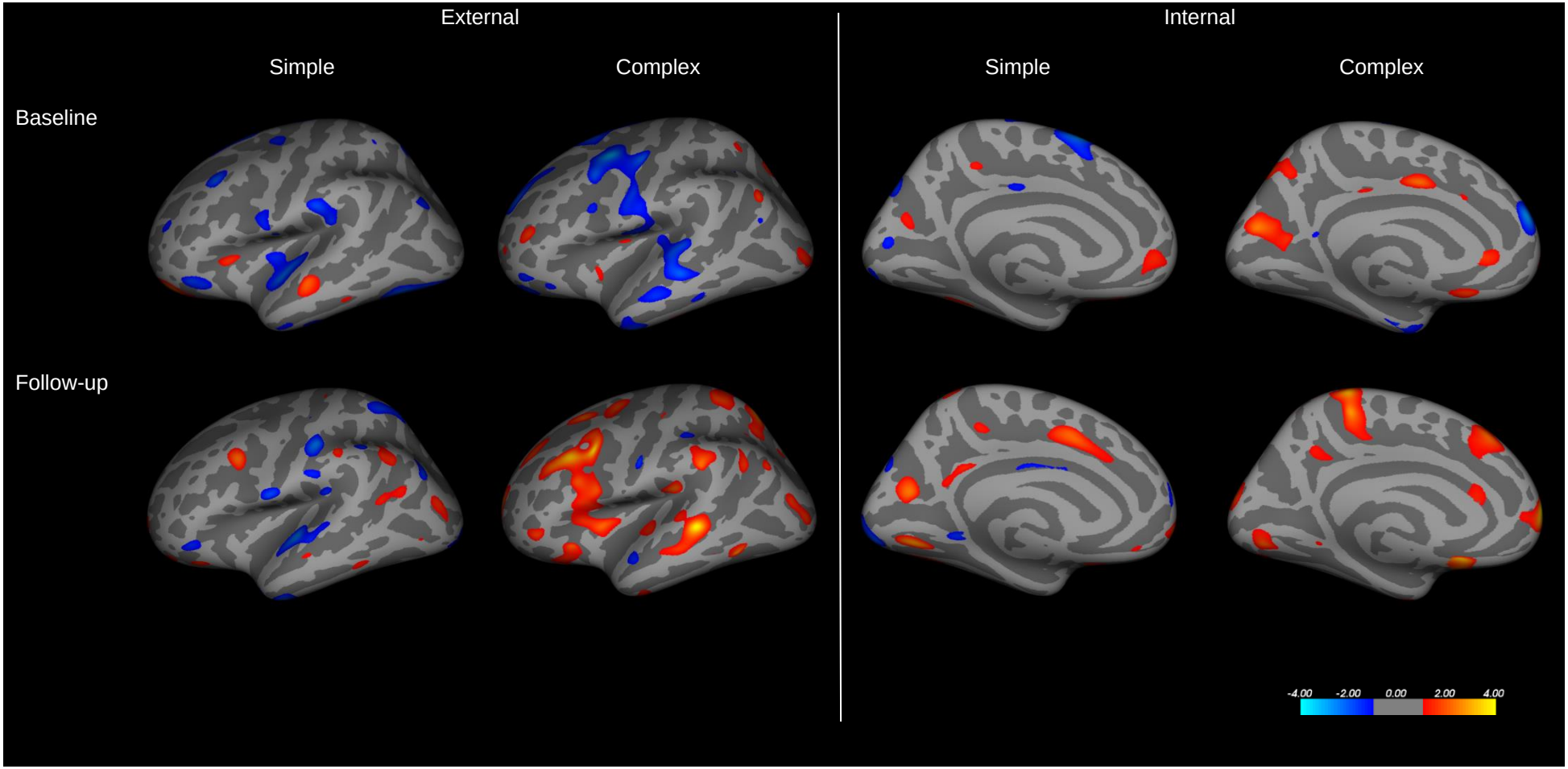


Figure 37: Comparison of cortical thickness in the left hemisphere between participants with RE and healthy controls at baseline and follow-up. Included are the outside and inside views of the left hemisphere. The top row consists of cortical thickness measures from baseline studies; the bottom row are measures from the follow-up. The simple contrast (left within the panel) examined direct differences in average thickness of vertices between groups, whereas the complex contrast (right within the panel) consisted of an age thickness correlation corrected for by sex. The colour bar ranges from p = 0.005 – 0.0001 in both a positive and a negative direction. In the simple contrast, red and yellow equate to a thicker cortex in the RE group compared to controls whereas in the blue/light blue represents a thinner cortex in the RE group compared to controls. For the complex contrast red and yellow reflects an increase in thickness with age-corrected for sex in the RE group compared to healthy controls, blue and light blue represent a

thinning of the cortex with age-corrected for sex. None of these results survived correction for family-wise error using Monte-Carlo null simulation (p=< 0.05).Degrees of freedom: 32.

Computational vertices-based analysis of the left hemisphere between the groups at baseline and at follow-up revealed multiple regions of difference ($p=<0.05$) however none of these regions survived correction for multiple comparisons using a Monte-Carlo null Z simulation.

Simple comparison baseline					
Cortical region	Significance	Size (mm²)	X	Y	Z
Superior frontal	-0.0016	731.06	-11.7	10.3	61.3
Lateral orbito frontal	0.004	740.45	-21	47	-13.3
Superior temporal	-0.004	726.32	-50.5	-9.1	-5.9
Middle temporal	0.0078	273.51	-49.5	-18.7	-14.3
Caudal middle frontal	-0.0087	214.06	-34.8	25.5	40.7
Fusiform	-0.0089	853.08	-40.9	-62.7	-12.5
Supramarginal	-0.014	427.67	-57.8	-24.5	24.6
Pars orbitalis	-0.02	271.25	-40.5	35.1	-12.6
Superior parietal	-0.02	252.65	-18.1	-81.3	37.4
Insula	0.029	82.51	-28.2	25.2	8.1
Inferior parietal	-0.034	137.25	-36.9	-77.5	29.9
Fusiform	0.036	149.06	-33.8	-49.2	-17.6
Medial orbito frontal	0.037	153.51	-14	44.9	-0.1
Pre-central	-0.038	109.1	-23.1	-9.3	48.3
Inferior temporal	-0.04	197.98	-46.4	-19.5	-30.5
Pre-central	-0.043	193.81	-56.4	5.2	18.6
Peri calcarine	-0.046	101.74	-8.8	-81.9	12
Pre-central gyrus	-0.046	129.78	-11.4	-21.3	73.5

Table 43: Simple comparison of cortical thickness in the left hemisphere between individuals with RE and healthy controls at baseline. Included are the regional areas the clusters are at their maximum, the p-value of the cluster, the blue shading indicates a thinner cortex in participants with RE when compared to healthy controls, the red shading indicates the reverse, the size of the area and the Talairach Montreal Neurological Institute (MNI) template coordinates (X, Y and Z) at the maximum difference for these regions. At baseline, a direct comparison between the two groups found mainly significant clusters of thinner cortex in the RE group. The most significant clusters were in the frontal and temporal regions.

Simple comparison follow-up					
Cortical region	Significance	Size (mm2)	x	y	z
Lingual	0.0015	457.15	-17	-71	-10
Superior temporal	-0.0029	563.08	-56.1	-10.9	0.8
Post-central gyrus	-0.0029	310.31	-51.8	-24	41.9
Cuneus	0.0055	362.3	-16.8	-67.8	14.5
Caudal middle frontal	0.0055	239.53	-35.3	10.3	33.5
Inferior temporal	-0.0061	349.86	-40.3	-6.8	-42
Superior frontal	0.0066	354.91	-10.5	7.2	41.1
Lateral occipital	-0.0099	881.78	-13.6	-93.2	-10.4
Frontal pole	0.011	314.26	-11.5	64.5	-3.9
Pre-central gyrus	-0.014	188.38	-57.6	-1.5	13.9
Fusiform	0.014	308.71	-38.1	-64.8	-16.8
Lateral orbito frontal	0.02	168.98	-16.2	16.4	-18.8
Temporal pole	-0.022	85.46	-33.1	15	-36.3
Superior parietal	-0.023	439.97	-28.3	-56.4	62.5
Inferior parietal	-0.023	180.13	-36.9	-77.5	29.9
Inferior parietal	0.028	179.44	-37.4	-79.2	12.5
Lingual	-0.029	90.74	-17.2	-44.5	-6.7
Inferior parietal	0.03	254.84	-49.3	-58.3	35
Lateral orbito frontal	0.03	80.12	-25.4	30.7	-11.1
Supramarginal	-0.033	99.05	-44.5	-31.4	18.3
Supramarginal	0.033	64.75	-54.2	-32.7	38.1
Supramarginal	-0.034	111.66	-49.5	-45.9	44.1
Superior parietal	0.035	86.57	-13	-53.3	65.6
Pars orbitalis	-0.037	129.47	-43.9	36.6	-14.2

Table 44: Simple comparison of cortical thickness in the left hemisphere between individuals with RE and healthy controls at follow-up. Included are the regional areas the clusters are at their maximum, the p-value of the cluster, the blue shading indicates a thinner cortex in the RE group compared to controls, the red shading indicates the reverse, the size of the area and the Talairach Montreal Neurological Institute (MNI) template coordinates (X, Y and Z) at the maximum difference for these regions. At follow-up, a comparison revealed a mixture of decreased and increased cortical thickness in participants with RE compared to controls. This appeared to be diffuse across the cortical surface.

The simple analysis revealed several areas of altered cortical thickness at baseline and follow-up; none of these clusters survived correction for multiple comparisons (Table 43 and Table 44). In the baseline cohort, thinner cortex was predominant. Clusters of highly significant thinner cortex were seen over the superior and caudal middle frontal, superior temporal and fusiform gyrus whereas a cluster of thicker cortex were seen over the lateral orbital frontal and middle temporal lobe. At follow-up, there was a greater number of clusters of both thicker and thinner cortex.

In the follow-up cohort, eight highly significant clusters were seen. Half were clusters of thicker cortex which were seen in the superior and caudal middle frontal, the cuneus and lingual gyrus. The other half were clusters of thinner cortex in the superior and inferior temporal, postcentral and lateral occipital regions. There were some similarities between the baseline and follow-up cohorts.

Region	Timepoint	X	Y	Z	Area (mm^2)
Superior temporal	Baseline	-50.5	-9.1	-5.9	726.32
	Follow-up	-56.1	-10.9	0.8	563.08
Superior frontal	Baseline	-11.7	10.3	61.3	731.06
	Follow-up	-10.5	7.2	41.1	354.91
Caudal middle frontal	Baseline	-34.8	25.5	40.7	214.06
	Follow-up	-35.3	10.3	33.5	239.53

Table 45: Left hemisphere cortical regions with clusters of altered cortical thickness at both baseline and follow-up. Included are cortical regions and the size of the area and the Talairach Montreal Neurological Institute (MNI) template coordinates (X, Y and Z) at the maximum difference for these regions. This table demonstrates that significantly different cortical regions are apparent at both the baseline and follow-up. These regions were found in the superior frontal, caudal middle frontal and superior temporal lobe.

Altered cortical thickness was seen in the superior frontal and temporal gyri and caudal middle frontal in both the baseline and follow-up cohorts (Table 45). The superior temporal lobe had a cluster of thinner cortex in both the baseline and follow-up cohorts. This cluster reduced in area between the two time points. Whereas the superior and caudal middle frontal regions contained clusters of thinner cortex in the baseline which were thicker at follow-up. In the superior frontal regions, the cluster was smaller in the follow-up cohort, whereas the caudal middle frontal cluster area was similar in area in the baseline and follow-up cohorts.

Complex Baseline					
Cortical region	Significance	Size (mm^2)	X	Y	Z
Caudal Middle Frontal	-0.00078	2128.15	-35.2	1	49.1
Superior Frontal	-0.0014	947.06	-7.8	56	22.9
Cuneus	0.003	829.44	-5.6	-71.9	16.6
Posterior Cingulate	0.0053	170.38	-6.6	-0.2	38.9
Middle temporal	-0.008	931.08	-48.3	-31.6	-9
Superior Parietal	0.01	481.09	-14.4	-76.5	45.5
Inferior temporal	-0.013	817.96	-39.4	-5.7	-42.4
Superior frontal	-0.014	540.3	-18.5	12.7	59.6
Inferior temporal	0.017	203.44	-46.6	-29.8	-23.3
Inferior parietal	0.02	92.02	-39.9	-68.3	33
Medial orbito frontal	0.02	120	-5.1	25.2	-19.9
Rostral middle frontal	0.021	134.21	-36.3	42	11.3
Rostral anterior cingulate	0.025	92.39	-3.7	32.6	1.1
Middle temporal	- 0.026	258.9	-58.8	-17.1	-17.4
Lateral orbito frontal	- 0.026	214.22	-21.9	40.6	-14.4
Pars orbitalis	-0.037	111.58	-35.8	46.2	-10
Lateral occipital	0.04	346.97	-28.4	-93.3	-2.7
Pars opercularis	0.043	45.47	-37.3	6	22.9

Table 46: Significant clusters of altered cortical thickness correlation with age in the left hemisphere in individuals with RE at baseline. Included are the regional areas the clusters are at their maximum, the p-value of the cluster, the blue shading indicates a decrease in cortical thickness correlated against age when compared to healthy controls, the red shading indicates the reverse, the size of the area and the Talairach Montreal Neurological Institute (MNI) template coordinates (X, Y and Z) at the maximum difference for these regions. At baseline, there was a large number of significant clusters which demonstrated an increase in cortical thickness with age in participants with RE compared to healthy controls.

Complex follow-up					
Cortical region	Significance	Size(mm²)	X	Y	Z
Banks of the superior temporal sulcus	0.00012	820.11	-48.1	-36.7	-0.7
Superior frontal	0.00013	796.65	-9.9	64.7	9.3
Caudal Middle Frontal	0.00028	2474.22	-36.8	9.7	35.3
Medial Orbito Frontal	0.00059	808.47	-6.1	22	-17
Paracentral	0.00075	694.98	-5	-42.4	67.1
Inferior temporal	0.0018	131.63	-50.2	-56.7	-11.3
Superior Parietal	0.003	555.34	-15.8	-67.1	57.5
Supramarginal	0.0048	343.52	-54.9	-39.5	33
Supramarginal	0.0054	221.37	-45.5	-27.6	21.4
Superior frontal	0.0056	466.52	-7.1	32.8	47.9
Superior frontal	0.0058	225.57	-23.8	15.3	50.1
Lingual	0.0083	498.42	-12.2	-81.7	-9.3
Superior frontal	0.012	237.34	-20.9	-1.2	48.6
Fusiform	0.013	293.54	-38.5	-6.5	-39
Superior Parietal	0.016	520.23	-26.6	-46.2	59.1
Cuneus	0.016	350.29	-10	-92	13.2
Rostral middle frontal	0.018	231.04	-19.3	40.8	33.7
Pars orbitalis	0.02	146.74	-43.8	40.2	-10.5
Post-central gyrus	-0.02	42.94	-49.9	-10.9	23.6
Lateral occipital	0.02	294.56	-26.3	-87.2	0.9
Inferior parietal	0.025	146.53	-45.8	-59.2	28.7
Transverse temporal	0.027	173.31	-51.7	-13.5	1.4
Inferior parietal	0.027	76.72	-42.1	-72.1	32
Superior temporal	-0.039	114.75	-54.5	-0.6	-14.1
Caudal anterior cingulate	0.044	73.3	-4.4	27.5	14.1
Post-central gyrus	0.044	14.93	-36.7	-24.8	44.6
Supramarginal	-0.048	63.67	-40.9	-28.8	36

Table 47: Significant clusters of altered cortical thickness in the left hemisphere in individuals with RE at follow-up. Included are the regional areas the clusters are at their maximum, the p-value of the area, the blue shading indicates a decrease in cortical thickness correlated against age when compared to healthy controls, the red shading indicates the reverse the size of the area and the Talairach Montreal Neurological Institute (MNI) template coordinates (X, Y and Z) at the maximum difference for these regions. At follow-up, the majority of the significant clusters demonstrated an increase in cortical thickness with age in participants with RE compared to healthy controls.

For the sake of brevity only highly significant clusters (p=<0.001) identified by the complex analysis will be discussed (Table 46 and Table 47), none of the clusters survived correction for multiple comparisons. Several clusters with apparent altered cortical development were seen in both the baseline and follow-up cohorts. In the baseline cohort clusters seen in the caudal middle frontal, superior frontal and middle temporal gyrus had a decrease in cortical thickness correlated with age,

whereas the cuneus and posterior cingulate had the reverse. At follow-up, an increase in cortical thickness in correlation with age predominated.

In the follow-up cohort, clusters were seen in the superior, caudal middle and medial orbitofrontal, inferior temporal and banks of superior temporal sulcus, paracentral, superior parietal and supramarginal and lingual gyrus. All these regions correlated demonstrated a correlation of increased cortical thickness with age. There were some regions which were seen in both the baseline and follow-up cohorts.

The superior and caudal middle frontal regions had clusters of altered cortex in both the baseline and follow-up cohorts (Table 48). In the baseline cohort, thickness of these regions decreased with age, whereas at follow-up, it was seen to increase. The clusters were within the same regions in both cohorts. The superior frontal cluster was larger in area the baseline cohort compared to the follow-up cohort, and caudal middle frontal cluster was greater in area in the follow-up cohort compared to the baseline cohort.

Cortical regions	Timepoint	X	Y	Z	Area
Superior frontal	Baseline	-7.8	56	22.9	947.06
	Follow-up	-9.9	64.7	9.3	796.65
Caudal middle frontal	Baseline	-35.2	1	49.1	2128.15
	Follow-up	-36.8	9.7	35.3	2474.22

Table 48: Left hemisphere cortical regions with clusters of altered cortical thickness correlation with age at both baseline and follow-up. Included are cortical regions and the size of the area and the Talairach Montreal Neurological Institute (MNI) template coordinates (X, Y and Z) at the maximum difference for these regions. There were two cortical locations which were significantly different at both baseline and follow-up; these were the superior frontal and caudal middle frontal regions.

Overall, within the baseline and follow-up cohorts, there were multiple regions of altered cortical development in the left hemisphere of participants with RE compared to healthy controls. In the baseline cohort, the simple comparison found clusters of thinner cortex in the superior frontal and caudal middle frontal regions, which became thicker compared to controls at follow-up. In RE, at baseline, a mixture of a decrease and increases in cortical thickness in correlation with age is seen whereas, at follow-up, thickening of cortex with age is the norm. The same regions had altered correlations with age at baseline where cortical thickness decreased with age, whereas at follow-up there was an increase in thickness with age.

6.4.2.3 *Right hemisphere*

6.4.2.3.1 Hypothesised regions

The average thickness for the hypothesised cortical regions is presented in Table 49. There was no significant difference between the hypothesised areas in either the baseline or follow-up cohort. MANOVA analysis found no significant difference in the corrected model in the baseline group ($p = 0.762$) whereas in the follow-up group there was a significance ($p = 0.028$) between the two groups, but this may be attributable to a type 1 error. At baseline, all the control regions had a thicker cortex than those in children with RE. Whereas, in the follow-up group, the pars triangularis, superior temporal gyrus, supramarginal gyrus and the inferior parietal region were thicker in controls compared to adolescents with RE. Furthermore, the pars opercularis and the transverse temporal region were thinner in controls compared to the RE group.

	Baseline				Follow-up			
	Control n=22	RE n=19	DF	Stats (p=)	Control n = 12	RE = 10	DF	Stats (p=)
Pars Triangularis (mm)	2.78 ± 0.14	2.73 ± 0.20	38	0.525	2.48 ± 0.17	2.45 ± 0.13	19	0.560
Pars Opercularis (mm)	2.84 ± 0.15	2.82 ± 0.16	38	0.876	2.62 ± 0.13	2.70 ± 0.13	19	0.538
Superior temporal gyrus (mm)	3.09 ± 0.16	3.03 ± 0.16	38	0.534	2.96 ± 0.17	2.92 ± 0.09	19	0.715
Transverse temporal regions (mm)	2.78 ± 0.18	2.71 ± 0.32	38	0.547	2.69 ± 0.19	2.78 ± 0.21	19	0.570
Supra-marginal gyrus (mm)	2.82 ± 0.11	2.81 ± 0.15	38	0.775	2.65 ± 0.13	2.61 ± 0.17	19	0.817
Inferior parietal region (mm)	2.77 ± 0.10	2.75 ± 0.13	38	0.455	2.59 ± 0.11	2.52 ± 0.17	19	0.574

Table 49: Right hemisphere average cortical thickness for hypothesised regions in controls and participants with RE at baseline and follow-up. Cortical regions defined from the Desikan-Killany atlas. Stats: Statistical analysis using MANOVA with covariates age and sex. DF: Degrees of freedom There was no significant difference between the hypothesised cortical regions between the RE group and healthy controls at both baseline and follow-up. .

6.4.2.3.2 Exploratory regions

	Baseline				
	Control n=22	RE n=19	DF	Stats	Effect size
Medial orbito-frontal gyrus (mm)	2.83 ± 0.13	2.91 ± 0.19	38	**0.037** [a]	0.49

*Table 50: Exploratory analysis of average cortical thickness in cortical regions within the right hemisphere. Presented are regions significant or close to significance. All regions are defined from the Desikan-Killany atlas. Values are in millimetres. Statistical analysis used MANOVA with age and sex as covariates. DF: Degrees of freedom **Bold** stats are significant. [a] did not survive Bonferroni correction. Reported effect size Cohens d. There was only one significant region identified by exploratory analysis. The medial orbitofrontal region was significantly thicker in participants with RE compared to healthy controls. This did not survive Bonferroni correction.*

Global analysis of the average thickness of cortical parcellation across the right hemisphere are presented in Table 50. At baseline, there was only one significant region the medial orbitofrontal gyrus, which was thicker in individuals with RE compared to controls. At follow-up, none of the regions showed a significant difference.

6.4.2.3.3 Cortical thickness maps

Vertices maps of cortical thickness in the right hemisphere are presented (Figure 38). At baseline, there is little difference between the two groups, other than a thinner cortex over the central sulcus and in the posterior cingulate. Evidence of thicker cortex over the superior frontal gyrus can be seen in the RE group. At follow-up, within the central sulcus, thinner cortex is prevalent in the control group whereas, in the RE group, this is patchy and in the inferior portion. Furthermore, the gyral ridges of the superior and middle temporal regions are not well defined and are thinner in the RE group compared to controls. Thin cortex under 1.5 mm can be seen in the lingual, peri-calcarine and precuneus cortical regions. A thin posterior cingulate is also seen; however, this is comparable in its extent between the two groups.

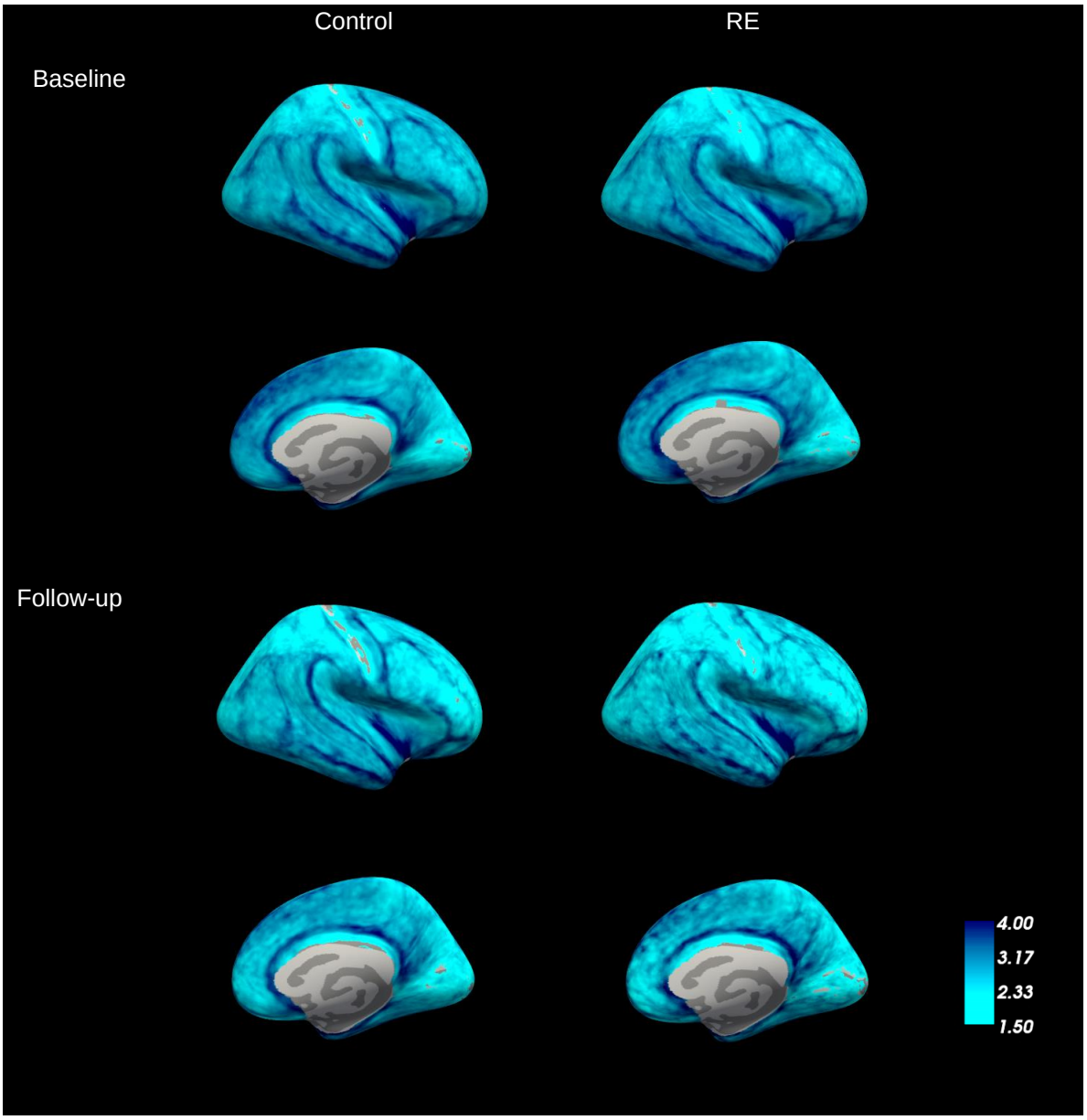


Figure 38: Cortical thickness maps of the right hemisphere in a composite of participants with RE and healthy controls. Average cortical thickness is displayed by a blue overlay over an inflated pial surface. Cortical thickness measures range from 1.5 mm (light blue) to 4 mm (dark blue). Any regions of the cortex which are less than 1.5 are in grey. Subcortical structures are excluded from the analysis. Casual analysis reveal little difference; however, many subtle differences can be observed.

6.4.2.3.4 Right hemisphere SPC vertices-based analysis

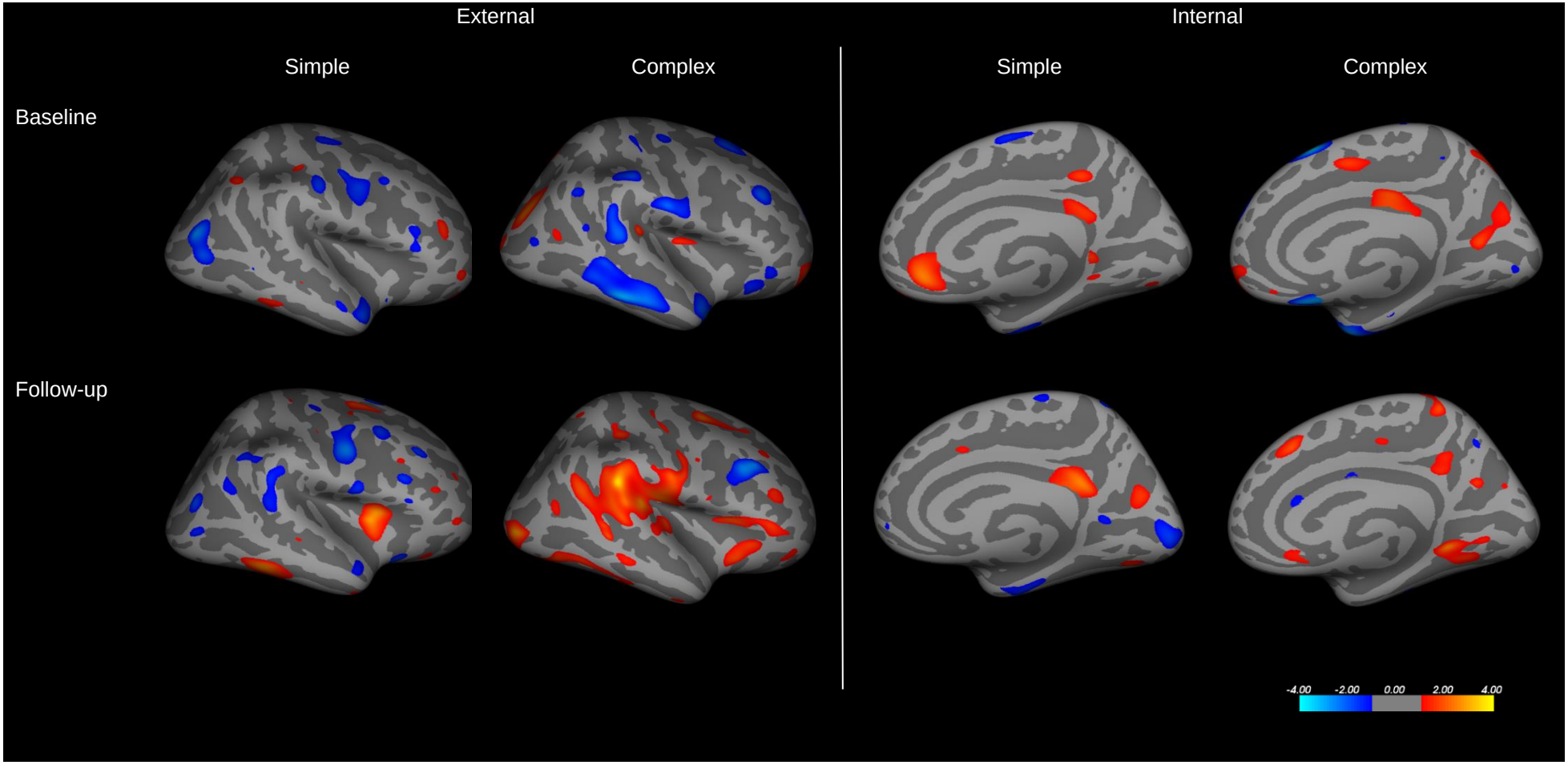


Figure 39: Comparison of right hemisphere cortical thickness between participants with RE and healthy controls at baseline and follow-up. The top row consists of cortical thickness measures from baseline studies; the bottom row are measures from the follow-up. The simple contrast examined direct differences in average thickness of vertices between groups whereas the complex contrast consisted of an age thickness correlation corrected for by sex. The colour bar ranges from p = 0.005 – 0.0001 in both a positive and a negative direction. In the simple comparison red and yellow equate to a thicker cortex in the RE group compared to controls whereas in the blue/light blue represents a thinner cortex in the RE group compared to controls. In the complex comparison red and yellow reflects an increase in thickness with age-corrected for sex in the RE group compared to healthy controls, blue and light blue represent a thinning of the cortex with age-corrected for by age. All results smoothed with 15 mm full-width height maximum kernel. The baseline results did not survive correction for family-wise error using Monte-Carlo null Z simulation (p=< 0.05).Degrees of freedom: 12.

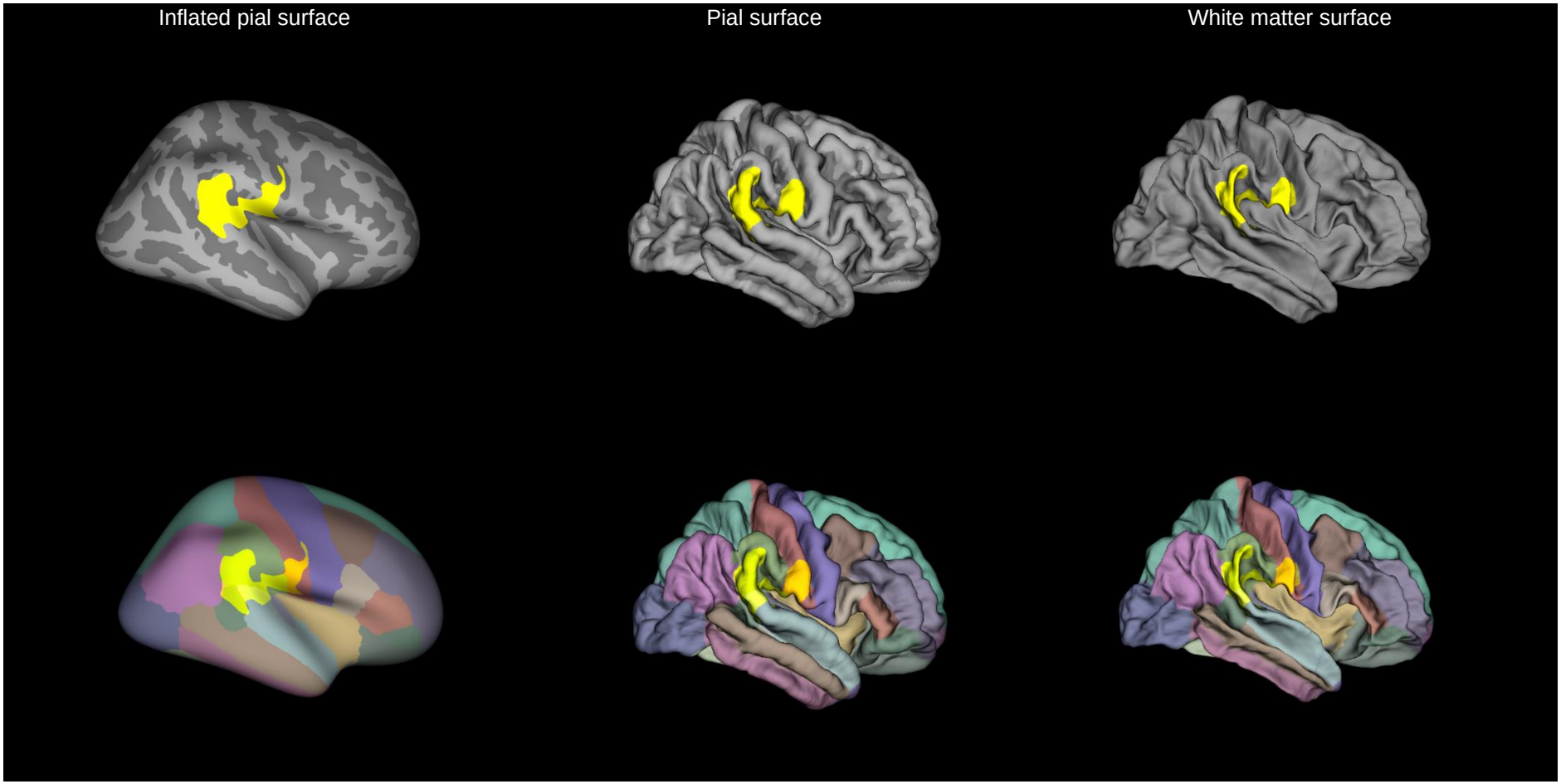


Figure 40: Difference in cortical thickness in the right hemisphere between participants with RE in seizure remission and healthy controls at follow-up. The panel is divided into an upper row of cortical models with the region of interest. The top row consists of different brain models, inflated pial, pial and white matter outlines. The lower row consists of the same models with a Desikan-Killany atlas overlay. The difference was calculated from a complex comparison of thickness-age correlation corrected for sex. This was then corrected for multiple comparisons using a Monte-Carlo simulation (p=0.05). The region of difference (yellow) represents an area where the cortical thickness increased with age in participants with RE, whereas in controls, there was a decrease in cortical thickness across age. The maximum significance of this region was in the post-central region. The region spreads between the lower portion of the post-central region, supramarginal gyrus, transverse temporal and the upper portion of the superior temporal region.

Simple comparison baseline					
Cortical region	Significance	Size (mm²)	Y	Y	Z
Inferior parietal	-0.0023	628.94	39.5	-77.4	14.8
Medial orbitofrontal	0.0031	372.27	11.1	35.3	-8.8
Lateral orbitofrontal	0.015	230.21	14.4	48.4	-19
Superior temporal	-0.016	406.51	48	8.7	-25.2
Precuneus	0.019	130.75	8.2	-40.4	42
Post-central gyrus	-0.019	174.95	53	-18.2	39.5
Pre-central gyrus	-0.02	519.38	55.5	-0.9	39
Isthmus cingulate	0.022	84.89	12.7	-45.9	1.9
Isthmus cingulate	0.023	159.58	8.6	-44.2	24.6
Rostral middle frontal	0.03	183.36	38.5	40.2	18
Superior frontal	-0.037	152.02	8.1	-3	63.4
Inferior parietal	0.038	129.79	42.8	-60.2	45.4
Fusiform	-0.039	240.96	35.5	-11	-34.7
Inferior temporal	0.04	138.17	55.5	-46.7	-20.4
Rostral middle frontal	0.043	90.78	36.1	54.7	-6.7
Pre-central gyrus	-0.043	204.99	32.6	-21.1	64
Post-central gyrus	0.047	59.38	42.4	-27.1	44.4

Table 51: Simple comparison of cortical thickness in the right hemisphere between individuals with RE and healthy controls at baseline. Included are the regional areas the clusters are at their maximum, the p-value of the cluster, the blue shading indicates thinner cortex when compared to healthy controls, the red shading indicates the reverse, the size of the area and the Talairach Montreal Neurological Institute (MNI) template coordinates (X, Y and Z) at the maximum difference for these regions. At baseline, there was a mixture of significant clusters which demonstrated either thicker or thinner cortex in respect to healthy control data. The most significant clusters were in the frontal, parietal and temporal regions.

Simple comparison follow-up					
Cortical region	Significance	Size (mm2)	X	Y	Z
Insula	0.0016	518.29	33.4	8.1	5.7
Inferior temporal	0.0025	720.64	53.2	-48.1	-18.8
Precentral	-0.0029	645.46	56.4	-1.4	36.9
Isthmus of cingulate	0.003	357.25	5.7	-45.5	24.4
Inferior parietal	-0.012	163.3	42.5	-76.5	15.2
Lateral orbitofrontal	-0.013	111.6	25.6	16.9	-17
Superior frontal	0.014	179.82	21.6	2.3	52.7
Superior frontal	-0.018	76.97	19.9	12.8	60.7
Peri-calcarine	-0.019	540.9	15	-89.3	3
Cuneus	0.02	333.14	16.9	-73	18.2
Superior parietal	-0.023	97.64	12.5	-51.9	64.5
Rostral middle frontal	-0.023	82.01	43.1	25.2	20.4
Caudal middle frontal	-0.024	218.03	41.7	16.9	47
Rostral middle frontal	-0.025	94.3	35.1	29	32.2
Precentral	-0.029	138.04	57.2	5.1	9.4
Lingual	0.03	149.46	25.9	-74	-6
Fusiform	-0.034	364.53	34	-14	-32
Supramarginal	-0.036	439.49	61.8	-38.1	24
Lateral occipital	-0.039	83.03	47	-79.4	3.7
Inferior parietal	-0.042	192.31	53	-50.7	39.6

Table 52: Simple comparison of cortical thickness in the right hemisphere between individuals with RE and healthy controls at follow-up. Included are the regional areas the clusters are at their maximum, the p-value of the cluster, the blue shading indicates a thinner cortex when compared to healthy controls, the red shading indicates the reverse, the size of the area and the Talairach Montreal Neurological Institute (MNI) template coordinates (X, Y and Z) at the maximum difference for these regions. At follow-up, significant clusters of thinner cortex are more prevalent in participants with RE compared to healthy controls.

In the right hemisphere at baseline, significant clusters were seen between the RE and control group. For the sake of brevity, only highly significant regions (p=<0.001) identified by the simple analysis will be discussed (Table 51 and Table 52). Compared to the left hemisphere, there was a smaller number of significant clusters in the baseline and follow-up cohorts. In the baseline, the inferior parietal region had a cluster of thinner cortex compared to controls, whereas the medial orbital frontal gyrus was thicker compared to controls. At follow-up clusters of thicker cortex predominated.

In the follow-up cohort, clusters of thicker cortex were seen in the insula, inferior temporal gyrus and isthmus of the cingulate. The pre-central gyrus contained a cluster of thinner cortex. Unlike the left hemisphere, there were no similar altered regions in the baseline and follow-up cohorts.

Complex comparison baseline					
Cortical region	Significance	Size(mm²)	X	Y	Z
Superior frontal	-0.00023	691.08	10.6	18.3	60.4
Middle temporal	-0.00093	1675.05	56.3	-34.6	-15.7
Medial orbito frontal	-0.00097	231.49	7.7	18.5	-20.6
Fusiform	-0.0018	1273.09	31.9	3.1	-41.4
Inferior Parietal	0.003	559.6	32.4	-76.9	16.3
Superior parietal	0.004	349.59	12.4	-71.6	52.4
Banks of the superior temporal sulcus	-0.006	489.15	62	-36.9	10.8
Post Central	-0.0069	533.8	62.2	-12.9	24.6
Rostral middle frontal	-0.008	277.61	37.6	29.1	31.4
Rostral middle frontal	0.011	803	18	59.3	-14.1
Posterior Cingulate	0.012	257.99	4.3	-19.6	32.3
Supramarginal	-0.012	306.71	52	-31.1	41.9
Superior temporal	0.013	94.26	46.6	-34	11.6
Precuneus	0.015	571.7	23.2	-63.4	9.3
Superior frontal	0.021	147.76	11.3	-1	50.2
Lateral occipital	0.036	164.01	23.1	-96.9	6
Precentral	-0.038	115.8	37.7	-17.4	63.9
Inferior parietal	-0.039	86.77	45.8	-56.4	34.8
Insula	0.04	109.65	33.6	-10.8	14.5
Lateral orbito frontal	-0.042	194.2	36.8	25.1	-17.1

Table 53: Significant clusters of altered cortical thickness in the right hemisphere in individuals with RE at baseline. Included are the regional areas the clusters are at their maximum, the p-value of the cluster, the blue shading indicates a decrease in cortical thickness correlated against age when compared to healthy controls, the red shading indicates the reverse the size of the area and the Montreal Neurological Institute (MNI) template coordinates (X,Y and Z) at the maximum difference for these regions. At baseline, the majority of the significant clusters represent a decrease in cortical thickness with age in the RE group compared to healthy controls.

Complex comparison follow-up					
Cortical region	Significance	Size(mm²)	X	Y	Z
Supramarginal	0.00024	4204.14	61.4	-39.7	23.7
Lateral occipital	0.0017	645.7	31.8	-89.7	-2.6
Rostral middle frontal	-0.0023	495.17	41	19.5	31.4
Lingual	0.0035	767.73	16.4	-45.8	-7
Superior frontal	0.0035	309.22	23.6	-1.4	46.4
Pars opercularis	0.011	567.01	39.9	19.1	10.2
Superior frontal	0.012	187.76	8.4	25.3	42.5
Precuneus	0.013	206.42	12.9	-45.9	63.9
Transverse temporal	0.016	293.75	44.2	-21.6	5.1
Lateral orbito frontal	0.0166	356.31	28.5	20	-5.9
Fusiform	0.018	817.12	45.2	-67.6	-13.9
Supramarginal	0.02	165.74	32.7	-33.8	40.1
Precuneus	0.026	77.61	13.2	-62.3	26.3
Isthmus cingulate	0.03	170.36	9	-46.5	30.7
Rostral middle frontal	0.034	166.07	38	34.6	19
Middle temporal	0.046	100.99	65.2	-31.8	-12.8
Precentral	0.049	45.72	32	-19.3	44.4

Table 54: Significant clusters of altered cortical thickness in the right hemisphere in individuals with RE at follow-up. Included are the regional areas the clusters are at their maximum, the p-value of the cluster, the blue shading indicates a decrease in cortical thickness correlated against age when compared to healthy controls, the red shading indicates the reverse, the size of the area and the Talairach Montreal Neurological Institute (MNI) template coordinates (X, Y and Z) at the maximum difference for these regions. All of the significant clusters demonstrated an increase in cortical thickness with age except for the rostral middle frontal region, which became thinner with age.

Follow-up corrected clusters					
Cortical region	Significance	Size(mm²)	X	Y	Z
Post-central gyrus	0.0001	2552.44	62.1	-9.6	28.9

Table 55: Significant clusters which survived correction for multiple comparisons of altered cortical thickness in the right hemisphere in individuals with RE at follow-up. There was one cluster with its maximal over the right post-central gyrus which survived correction for multiple comparisons. This cluster demonstrated an increase in cortical thickness in the RE group with age compared to healthy controls.

In the complex comparison at baseline, there were highly significant clusters within the superior, rostral middle and medial orbitofrontal, middle temporal, banks of the superior temporal sulcus and fusiform gyrus and post-central gyrus (Table 53). These regions demonstrated a decrease in cortical thickness with age when compared to controls, whereas clusters which revealed a positive correlation between age and cortical thickness were seen in the inferior and superior parietal regions. In the follow-up group, there were fewer highly significant clusters.

In the follow-up group, there was a reduction in highly significant clusters, and in those clusters, the predominant trend was for an increase in cortical thickness with age compared to healthy controls (Table 54). Highly significant clusters, with an increase in cortical thickness with age, were seen in the supramarginal gyrus, lateral occipital and lingual gyrus and superior frontal gyrus. The rostral middle frontal gyrus revealed a decrease in cortical thickness with age compared to controls. There were two regions which were reported in both the baseline and follow-up cohorts.

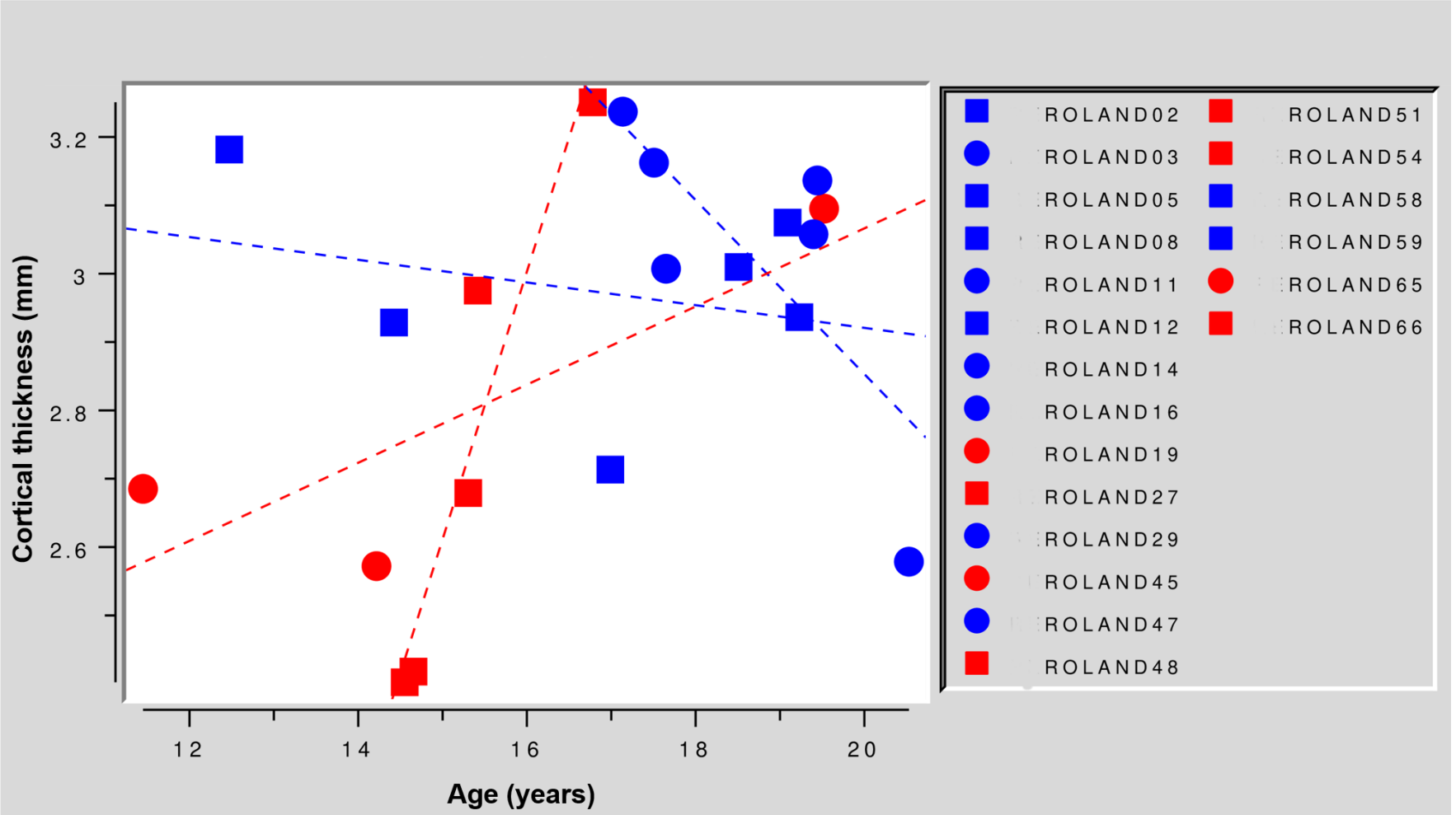


Figure 41: Graph of cortical thickness in the right post-central gyrus in individuals with RE compared to healthy controls at follow-up. The right-side panel includes the participant's codes. RE group in red and healthy controls in blue. Squares points are males and circles females. Included are regression lines for sex. Age of participant at scan in years. Cortical thickness in mm. This graph demonstrates a difference in cortical thickness between participants with RE and healthy controls. The majority of the RE group has a thinner cortex, less than 3 mm compared to the healthy controls who mainly have a cortical thickness greater than 3mm.

Cortical regions	Timepoint	X	Y	Z	Area (mm^2)
Superior frontal	Baseline	10.6	18.3	60.4	691.08
	Follow-up	23.6	-1.4	46.4	187.76
Rostral middle frontal	Baseline	37.6	29.1	31.4	277.61
	Follow-up	41	19.5	31.4	495.17

Table 56: Right hemisphere cortical regions with clusters of altered cortical thickness correlation with age at both baseline and follow-up. Included are cortical regions and the size of the area and the Montreal Neurological Institute (MNI) template coordinates (X, Y and Z) at the maximum difference for these regions. Two significant clusters were apparent at both baseline and follow-up. These included the superior frontal region and the rostral middle frontal region.

The superior and rostral middle frontal regions in individuals with RE had altered age-cortical thickness correlations compared with controls in the baseline and follow-up cohorts (Table 56). The rostral middle frontal regions contained clusters of decreased cortical thickness with age in the baseline and follow-up cohorts. These clusters were within similar locations, but there was a larger cluster area in the follow-up cohort compared to the baseline cohort. Whereas the superior frontal regions contained clusters which had a negative correlation between age and cortical thickness in the baseline cohort, and the correlation was positive in the follow-up. The cluster also appeared to be in a different location between the two time points and larger in area at baseline compared to the follow-up cohort. Correction for multiple comparisons revealed a large cluster of increased thinning in correlation with age in the follow-up cohort.

Correction for multiple comparisons revealed a large cluster with peak significance in the post-central gyrus (Table 55). This cluster had an area of 2552.44 mm^2 and included parts of the supramarginal gyrus, inferior parietal lobe, banks of the superior temporal sulcus and superior temporal gyrus. This cluster revealed a correlation between an increase in age with an increase in cortical thickness within these regions. The graph Figure 41, demonstrates the data points for this cluster. The majority of the RE group appears to have a reduction in cortical thickness below 3 mm whereas the majority of the control group has a cortical thickness greater than 3 mm. Overall, there the SPC analysis of the right hemisphere reveals altered cortical thickness around the right pre and post-central gyrus in individuals with RE.

6.4.2.4 *Cross-sectional subcortical volumes*

6.4.2.4.1 Intra-cranial volume and its constituents

To place the analysis of subcortical volumes into context, the findings for intracranial volume (ICV), total grey matter (GM), total white matter (WM) volume, the volume of non-WM or GM tissue and the ratio between GM and WM are presented. The volume of non-GM or WM tissue was calculated by ICV-(GM+WM), and the ratio was calculated by dividing GM by WM. Significant differences were seen in the ICV and non-GM or WM volume in the baseline cohort.

In the baseline, RE cohort a significantly larger mean ICV ($p = 0.0026$) and smaller non-GM or WM volume ($p = 0.018$) with large variance was recorded. Multiple analysis of variance (MANOVA) of the volumes with the following covariates, age at scan and sex found no significance between groups ($F=0.978$, $p=0.432$). The covariate sex appeared to affect differences in ICV ($p=0.027$) significantly, but this was not the case for non-GM or WM volume. Furthermore, age had a significant influence on GM ($p=0.033$), ICV ($p=0.024$) and non-GM or WM volume ($p=0.007$) there was a trend towards significance for the ratio of GM and WM ($p=0.097$). Post-hoc analysis of variance (ANOVA) found no significance between the groups for any of the variables. A similar, pattern of results was seen in the follow-up cohort.

In the follow-up cohort, the RE group had significantly larger mean ICV ($p = 0.011$) and non-GM or WM volumes ($p=0.044$). MANOVA statistical analysis with age at scan and sex as covariates revealed no significant difference between the groups ($p=0.217$). Post-hoc analysis revealed ICV ($p=0.004$) and non-GM/WM ($p=0.008$) to be significant for sex but not for group ($p=0.208$, 0.458) or age ($p=0.332$, 0.459). To further explore the relationship between ICV and non-grey or white matter volumes, Pearson correlations were performed.

	Baseline				Follow-up			
	RE = 19 (mean±sd)	Control = 22 (mean±sd)	DF	Stats (p=)	RE = 10 (mean±sd)	Control =12 (mean±sd)	DF	Stats (p=)
Intra-cranial volume (mm^3)	1544581.58 ± 136638.27	1542471.82 ± 101631.35	38	**0.026**	1594702.00 ± 125286.22	1507527.50 ± 122025.171	19	**0.011**
Total grey matter volume (mm^3)	787323.86 ± 33966.77	782618.90 ± 31018.35	38	0.127	733179.13 ± 25503.13	706356.75 ± 35677.68	19	0.309
Total white matter volume (mm^3)	448314.97 ± 20114.02	450563.28 ± 13763.92	38	0.957	451217.03 ± 26660.88	442617.38 ± 21050.11	19	0.425
Non-GM or WM volume (mm^3)	308942.75 ± 148388.67	309289.64 ± 99063.59	38	**0.018**	410306.14 ± 125641.19	358552.52 ± 143549.77	19	**0.044**
Ratio GM/WM	1.76	1.74	38	0.308	1.63	1.60	19	0.463

Table 57: Mean intracranial volume, grey and white matter volumes in children with RE and healthy controls. There were two volumes which were significantly different at both baseline and follow-up, which were the intracranial and the non-grey or white matter volumes. Stats calculated using MANOAVA with sex and age as covariates. DF: Degrees of freedom.

Pearson correlations in both the baseline and follow-up cohorts, revealed very strong correlations between ICV and non-grey or white matter volumes (Figure 42). In the RE group, the correlations were 0.93 in the baseline and 0.924 in the follow-up. In the control group, they were 0.901 in the baseline and 0.928 in the follow-up. These findings would suggest that there is no difference in the developmental relationship between ICV and non-grey or white matter volumes in individuals with RE and healthy controls.

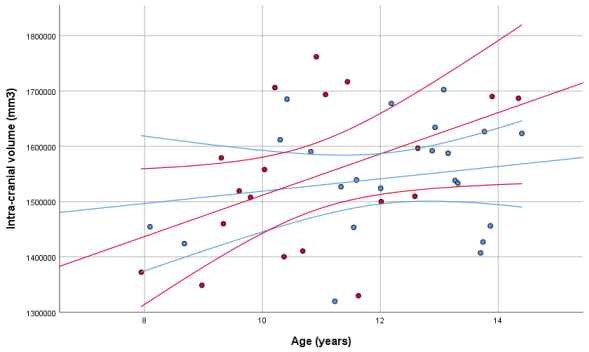
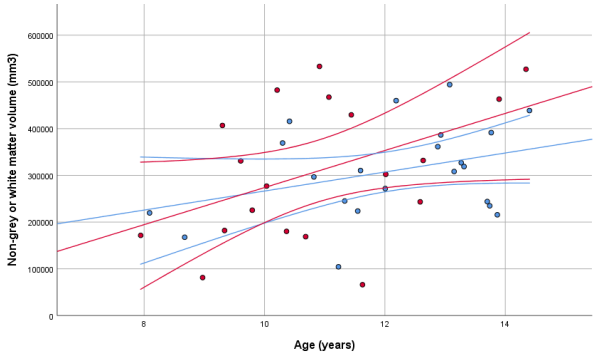
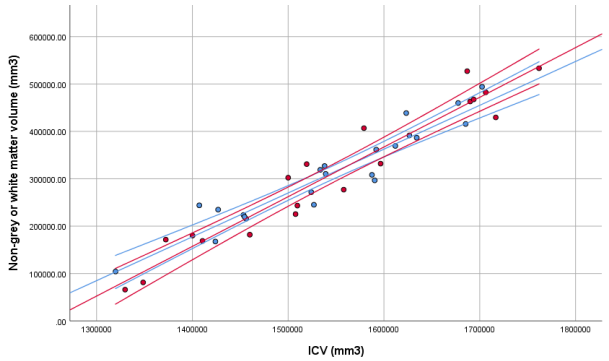
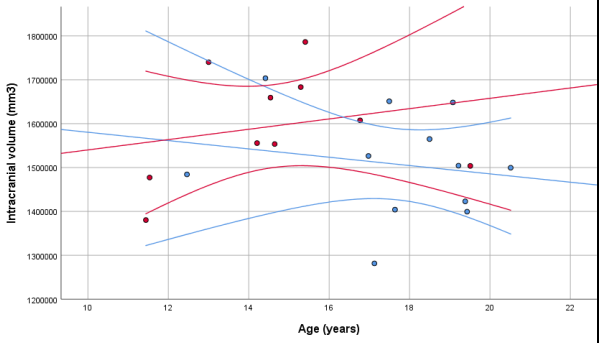
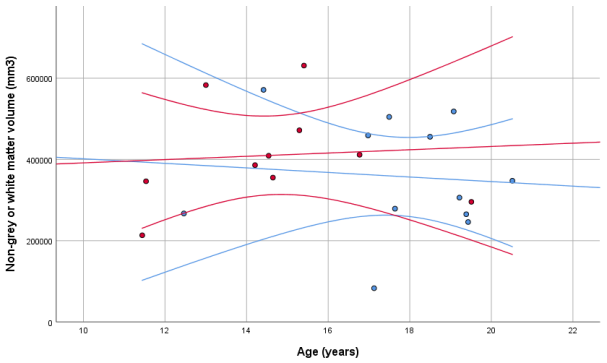
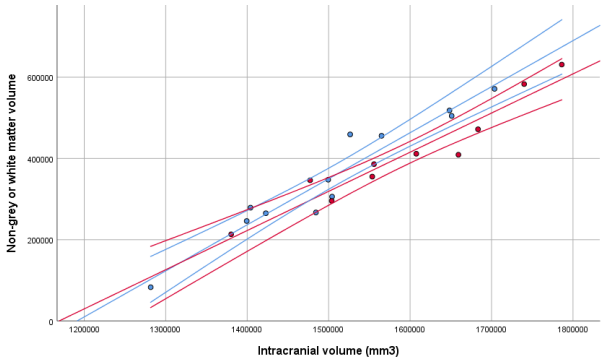
	Intra-cranial volume	Non-grey or white matter volume	Intra-cranial volume and non-grey or white matter volume
Baseline			
Follow-up			

Figure 42: The correlation of ICV and non-grey or white matter volumes and age in individuals with RE and healthy controls. RE (red) and controls (blue). Lines of best fit with 95% confidence intervals. These graphs demonstrate evidence for an altered increase in intracranial volume (ICV) and non-grey and white matter volumes in participants with

RE and healthy controls. This is most apparent in the baseline compared to the follow-up data. The panels on the right of the image demonstrate that there is a linear relationship between ICV volume and non-grey and white matter volumes.

6.4.2.4.2 The volume of subcortical structures

At baseline and follow-up, there was no significant difference between the volumes of any of the subcortical structures (Table 58). A MANOVA model revealed no significant difference between the groups. Furthermore, there were no trends toward significance. Post-hoc ANOVA for each factor revealed no significant findings.

	Baseline				Follow-up			
	RE (n=19)	Control (n=22)	DF	Stats (p=)	RE (n=10)	Control (n=12)	DF	Stats (p=)
Left Thalamus	7581.59 ± 615.22	7692.41 ± 556.37	38	0.943	7646.88 ± 794.04	7380.86 ± 448.04	19	0.445
Right Thalamus	7013.46 ± 588.70	7173.37 ± 376.88	38	0.630	6997.07 ± 329.34	6786.93 ± 353.76	19	0.331
Left Putamen	5455.32 ± 735.01	5384.14 ± 720.50	38	0.822	5614.80 ± 437.61	5501.21 ± 643.33	19	0.899
Right Putamen	5622.05 ± 608.48	5684.31 ± 581.01	38	0.613	5705.06 ± 459.64	5551.24 ± 547.35	19	0.772
Left Caudate	3850.89 ± 339.33	3963.98 ± 344.23	38	0.448	3657.00 ± 245.90	3773.75 ± 297.82	19	0.754
Right Caudate	4023.933 ± 352.89	4096.34 ± 336.25	38	0.586	3837.08 ± 268.49	3927.94 ± 319.16	19	0.477

Table 58: Average volume of subcortical structures in individuals with RE and controls at baseline and follow-up. Volume in mm³. Stats: MANOVA test with age and sex as covariates. There was no significant difference between the volumes of the subcortical structures between participants with RE and healthy controls.

6.4.3 **Longitudinal changes in parcellated brain regions**

A priori hypothesised areas were analysed with respect to the rate of change in cortical thickness and SPC; these were the same cortical regions which were explored in the cross-sectional analyses and included, pars triangularis, pars opercularis, superior temporal gyrus, transverse temporal region, supramarginal gyrus and inferior parietal regions. Furthermore, a global analysis of rate and SPC was performed for each hemisphere and an exploratory analysis of each cortical parcellation within the hemisphere.

6.4.3.1 *Interhemispheric comparison*

	RE (n =8)		Control (n = 12)				
	Rate (mm/year)	SPC (%)	Rate (mm/year)	SPC (%)	DF	Rate (p =)	SPC (p =)
Left hemisphere	-0.03 ± 0.03	-1.28 ± 1.11	-0.03 ± 0.02	-1.03 ± 0.63	17	0.806	0.836
Right-hemisphere	-0.05 ± 0.04	-1.66 ± 1.39	-0.03 ± 0.02	-1.16 ± 0.69	17	0.633	0.659

Table 21: Changes in bi-hemisphere cortical thickness in individuals with RE between active epilepsy and seizure remission. MANOVA corrected for age and sex was used to identify statistical significance. DF: Degrees of freedom. There were no significant differences in the longitudinal changes in hemispheric cortical thickness between participants with RE and healthy controls.

The average change in cortical thickness in each hemisphere as measured by the rate of change and SPC would suggest that in both individuals with RE and healthy controls there is a decrease in cortical thickness (Table 21). A MANOVA model found that this decrease was not significant. Even though the rate of cortical change was similar, there were larger changes in cortical thickness in the RE group compared to healthy controls.

6.4.3.2 *Left hemisphere*

6.4.3.2.1 Hypothesised regions

In both groups across all the hypothesised regions, there were decreases in average cortical thickness. However, there was no significant difference between the group in either the rate (p=0.832) or SPC (p= 0.877) (Table 59). The rate of cortical thinning was matched between the control and RE group in the pars opercularis, transverse temporal region and inferior parietal region. Cortical thinning measured by SPC found little difference in change in the pars opercularis, over double the amount of thinning in the transverse temporal region and reduced thinning in the inferior parietal region in the RE group compared to controls. Whereas for the pars triangularis, superior temporal gyrus and supramarginal gyrus, there was more thinning in the RE group compared to controls, which translated into a greater change in cortical thickness as measured by SPC.

	RE n = 8		Control n = 12				
	Rate (mm/year)	SPC (%)	Rate (mm/year)	SPC (%)	DF	Rate stats (p=)	SPC stats (p=)
Pars Triangularis	-0.05 ± 0.02	-1.97 ± 0.72	-0.04 ± 0.02	-1.55 ± 0.87	17	0.506	0.587
Pars Opercularis	-0.03 ± 0.02	-1.05 ± 0.63	-0.03 ± 0.03	-1.00 ± 1.02	17	0.376	0.410
Superior temporal gyrus	-0.04 ± 0.07	-1.22 ± 2.21	-0.01 ± 0.02	-0.37 ± 0.80	17	0.710	0.701
Transverse temporal region	-0.01 ± 0.03	-0.44 ± 1.28	-0.01 ± 0.02	-0.20 ± 0.81	17	0.949	0.962
Supra-marginal gyrus	-0.04 ± 0.04	-1.26 ± 1.48	-0.03 ± 0.02	-0.98 ± 0.72	17	0.919	0.928
Inferior parietal region	-0.04 ± 0.03	-1.38 ± 0.96	-0.04 ± 0.02	-1.60 ± 0.70	17	0.138	0.152

Table 59: Changes in cortical thickness within hypothesised regions in participants with RE and controls.

Included are the rate of change (mm/year) and symmetrised per cent change. Stats: MANOVA with age and sex as covariates. DF: Degrees of freedom. There was no significant difference between the hypothesised regions in the participants with RE and healthy controls.

Exploratory analysis

	RE n = 8		Control n = 12			
	Rate (mm/year)	SPC (%)	Rate (mm/year)	SPC (%)	Rate stats (p=)	SPC stats (p=)
Caudal anterior cingulate	0.006 ± 0.058	0.17 ± 1.92	-0.03 ± 0.03	-1.04 ± 1.13	0.023	0.023
Caudal middle frontal region	-0.04 ± 0.03	-1.40 ± 1.08	-0.038 ± 0.03	-1.33 ± 1.15	0.046	0.043

Table 60: Left hemisphere cortical regions with a significant difference in change in cortical thickness compared to controls between baseline and follow-up.

Stats: MANOVA model with age and sex as covariates. An exploratory analysis found the caudal anterior cingulate and caudal middle frontal region to be significantly different in the participants with RE. In the participants with RE, the caudal anterior cingulate increased with age, whereas in controls it decreased and in the caudal middle frontal region there was a greater reduction in cortical thickness compared to controls.

Global MANOVA analysis of the average SPC change in cortical thickness (hypothesised groups excluded) per cortical region found no difference between the two groups (p= 0.624). In the corrected model, the caudal anterior cingulate (P=0.023) and caudal middle frontal regions (p=0.043) were significant, but these findings did not survive Bonferroni correction (Table 60). Both of these regions had reduced rates of cortical thinning in participants with RE compared to healthy controls. Interestingly, despite a low rate of thinning in the caudal middle frontal region, the overall change in cortical thickness was greater in the caudal middle frontal region, indicating a thinner cortex at baseline in the RE group. Post-hoc ANOVA of the global data found sex to be significant for inferior-parietal thickness and average age significant for the caudal anterior cingulate and middle frontal region.

6.4.3.3 *Right hemisphere*

6.4.3.3.1 Hypothesised regions

	RE n = 8		Control n = 12				
	Rate (mm/year)	SPC (%)	Rate (mm/year)	SPC (%)	DF	Rate stats (p=)	SPC stats (p=)
Pars Triangularis	-0.05 ± 0.02	-1.91 ± 0.75	-0.04 ± 0.02	-1.54 ± 0.81	17	0.730	0.760
Pars Opercularis	-0.06 ± 0.04	-2.30 ± 1.49	-0.03 ± 0.03	-1.20 ± 1.11	17	0.262	0.299
Superior temporal gyrus	-0.05 ± 0.07	-1.80 ± 2.70	-0.02 ± 0.02	-0.70 ± 0.69	17	0.524	0.536
Transverse temporal region	-0.02 ± 0.05	-0.83 ± 1.68	-0.003 ± 0.02	-0.12 ± 0.83	17	0.612	0.663
Supra-marginal gyrus	-0.06 ± 0.03	-1.99 ± 1.09	-0.04 ± 0.02	-1.32 ± 0.82	17	0.471	0.488
Inferior parietal region	-0.05 ± 0.03	-1.89 ± 1.22	-0.04 ± 0.03	-1.60 ± 0.99	17	0.453	0.502

Table 61: Right hemisphere cortical regions with a significant difference in change in cortical thickness compared to controls.

Stats: MANOVA model with age and sex as covariates. DF: Degrees of freedom There was no significant difference between the hypothesised regions in the participants with RE and healthy controls.

In the hypothesised regions within the right hemisphere, there was a decrease in cortical thickness in both groups; this appeared to be at the greatest rate in the RE group compared to the controls (Table 61). Despite, the difference in cortical thinning, the MANOVA model was not significant, and individual regions were not significant.

6.4.3.3.2 Exploratory analysis

The exploratory analysis in the right hemisphere did not identify any significant regions

6.4.4 **Longitudinal changes in SPC vertices analysis**

6.4.4.1 *Controls*

In this section, the longitudinal data are presented for symmetrised per cent change (SPC) in cortical thickness when measured by vertices. This section is divided into control, RE and a comparison between the two groups. Only participants who were scanned at baseline and follow-up with neuroimaging data of quality suitable for analysis were included in this section.

In the control group, there was a differing amount of statistically significant cortical thinning between the two hemispheres (Figure 43). In the right hemisphere, cortical thinning was widespread both in the inner and outer hemisphere. Despite this, small regions of reduced thinning were seen in the para-central, superior pre and post-central gyri. Within the hemisphere, the peri-calcarine and lingual regions also had reduced thinning. A similar picture was seen in the left hemisphere; however, regions of non-thinning were expansive.

In the left hemisphere there appeared to be a band of reduced thinning of the cortex which ranged from the paracentral regions and down the anterior post-central gyrus, central sulcus, precentral gyrus and part of the posterior superior frontal region. This region extended into the anterior component of the superior, middle and inferior temporal gyrus. Within the hemisphere, the peri-calcarine and lingual regions also had reduced thinning.

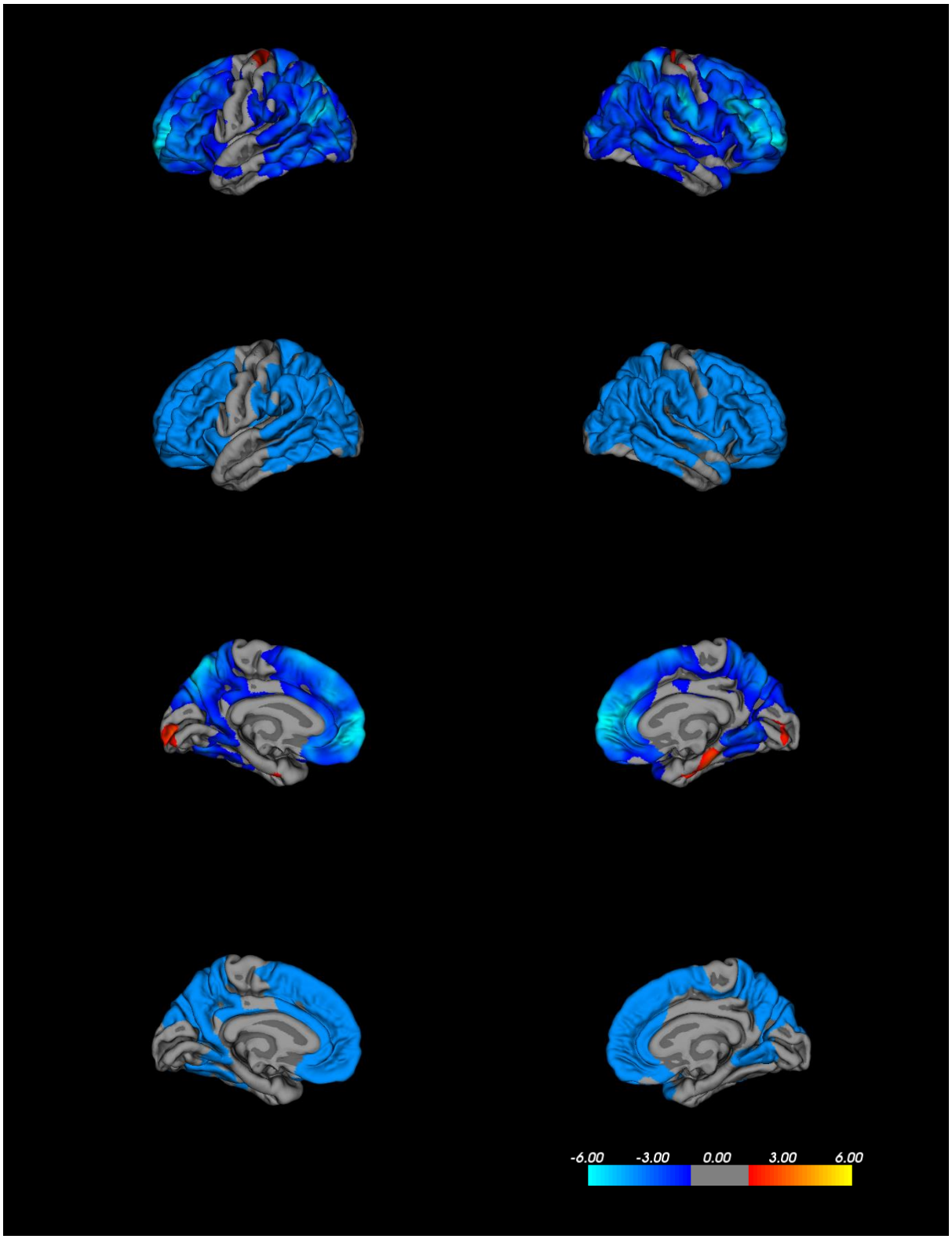


Figure 43: Longitudinal changes in cortical thickness in healthy controls. The statistical maps indicate if average changes in cortical thickness were greater than zero. Left column (left hemisphere) and right column (right hemisphere). The first and second rows external surface of the cortex. Third and fourth rows internal surface of the cortex. The first and third rows are uncorrected, whereas the second and fourth are corrected for multiple comparisons (Monte-Carlo simulation, p= 0.05). Blue/light blue represents a decrease in cortical thickness with age (p=-0.05-0.000001). Red and yellow an increase in cortical thickness with age (p=0.005-0.000001). None blue areas in corrected images could represent reduced thinning or thickening of the cortex.

6.4.4.2 *Rolandic Epilepsy*

In the RE group, statistically significant thinning in both hemispheres was seen to a lesser extent than what was seen in the control group (Figure 44). As a result, the areas where significant thinning occurred are described. In the outer left hemisphere, describing anterior to posteriorly, thinning was seen over regions of the superior and rostral frontal areas. A small region in the posterior part of the post-central gyrus had cortical thinning. Thinning was also seen in the posterior part of the superior, middle and inferior temporal gyri and parts of the superior and inferior parietal lobe. Within the hemisphere, thinning was seen anteriorly in parts of the superior frontal medial orbitofrontal and rostral anterior cingulate and posteriorly in the precuneus. A similar pattern of cortical thinning was seen in the right hemisphere.

In the right hemisphere, thinning in parts was like a mirror image of the left hemisphere; however, it was less patchy and covered a smaller area. In the outer hemisphere, thinning was seen anteriorly in the superior, rostral middle, lateral orbital and caudal middle frontal regions. In the posterior regions, there is a “C” shape of thinning which starts at part of the posterior inferior post-central gyrus and curves across parts of the supra-marginal, superior parietal and inferior parietal. It ends at the posterior component of the middle temporal gyrus. Within the hemisphere, thinning was seen anteriorly in parts of the superior frontal regions and the rostral and caudal anterior cingulate and posteriorly, in the precuneus.

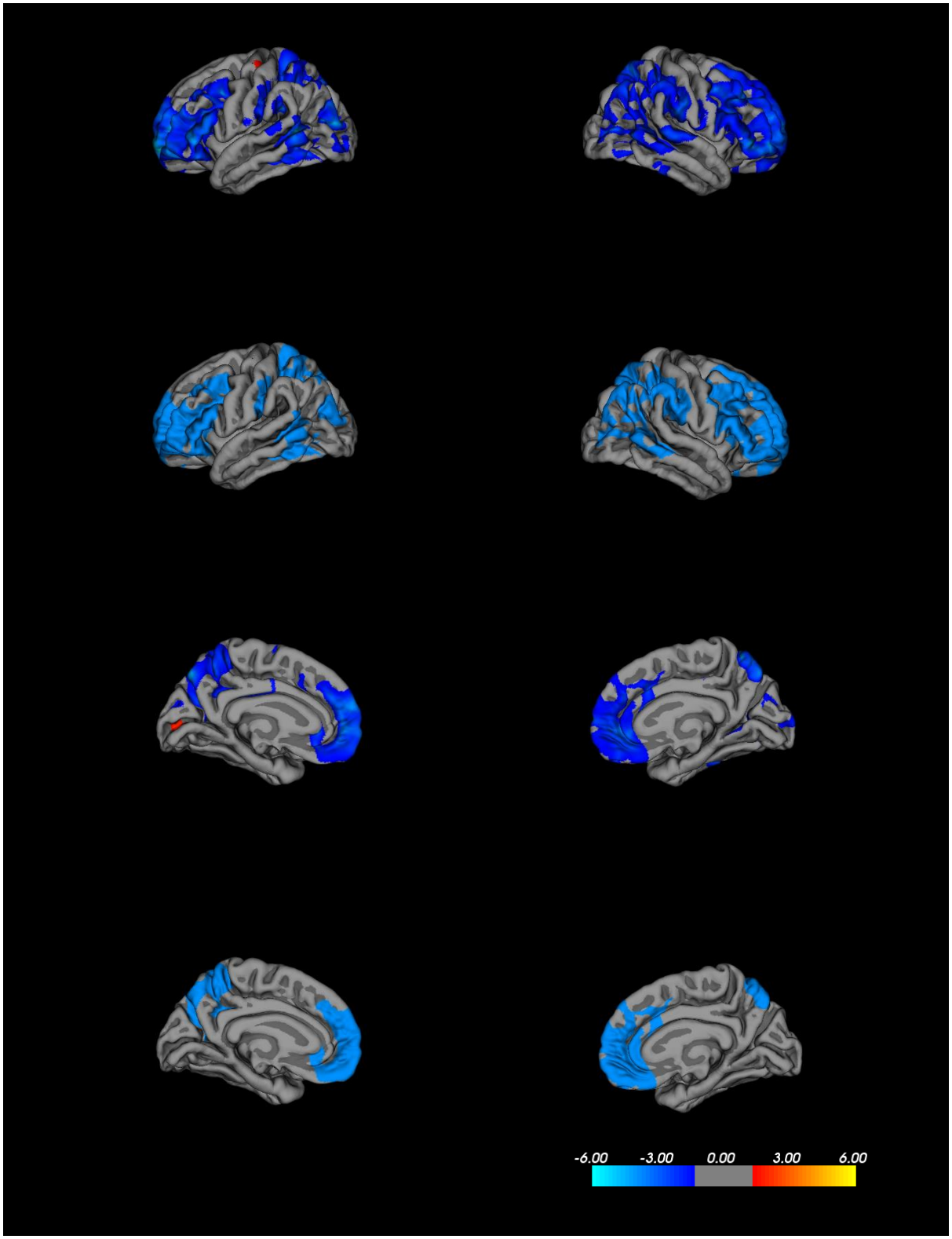


Figure 44: Longitudinal changes in cortical thickness in participants with RE. The statistical maps indicate if average changes in cortical thickness were greater than zero. Left column (left hemisphere) and right column (right hemisphere). The first and second rows external surface of the cortex. Third and fourth rows internal surface of the cortex. The first and third rows are uncorrected, whereas the second and fourth are corrected for multiple comparisons (Monte-Carlo simulation, p= 0.05). Blue/light blue represents a decrease in cortical thickness with age (p=-0.05-0.000001). Red and yellow increase in cortical thickness with age. (p=0.05-0.000001).

6.4.4.3 *Left hemisphere longitudinal comparison of changes in cortical thickness between rolandic epilepsy and control group*

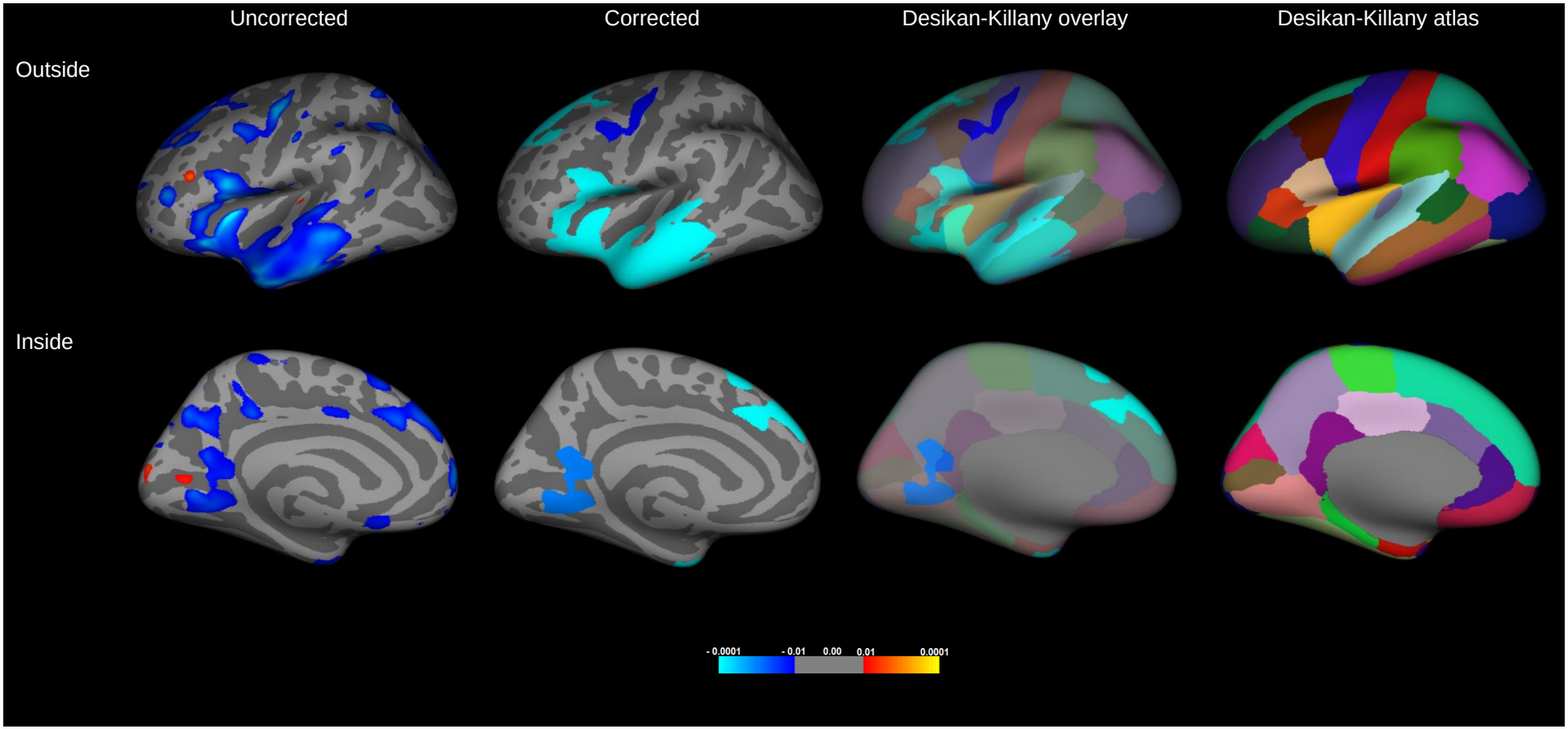


Figure 45: Differences in changes in cortical thickness in the left hemisphere between participants with RE and healthy controls between baseline and follow-up. The top row is the outer hemisphere, and the bottom row is the inner hemisphere. From left to right: First column uncorrected inflated pial model, second column; inflated pial corrected model, third column; inflated pial with Desikan-Killany overlay and fourth column Desikan-Killany atlas on an inflated pial model. Analysis corrected for age and sex. All results are corrected for familywise error using a Monte-Carlo simulation (p=0.05). Clusters colours in the first column reflect the level of significance ranging from p=-0.0001 (blue) to 0.0001 (red). Blue clusters represent a decrease in cortical thinning with age compared to the control group which increased in cortical thinning with age. Cluster colours relate to the p-value of the entire cluster. Degrees of freedom = 12.

Left hemisphere					
Cortical region	Significance	Size(mm²)	X	Y	Z
Insula	-0.0000023	8322.78	-29.4	14.1	7.8
Precentral	-0.0003	928.66	-43.5	-8.1	52.5
Superior frontal	-0.001	310.22	-8.8	62.3	8.9
Rostral middle frontal	-0.0017	218.42	-37.1	42.6	10
Inferior parietal	-0.0018	285.56	-33.2	-58.9	37.3
Rostral middle frontal	0.0024	63.56	-41.7	30.6	20.1
Rostral middle frontal	-0.0029	2299	-26.6	34	33.9
Postcentral	-0.0042	63.2	-56.9	-16.3	41.3
Precuneus	-0.0044	539.87	-10.9	-70	38.3
Lingual	-0.0045	1420.54	-5.9	-67.7	2.4
Precuneus	-0.0059	266.72	-8.2	-38.4	38.2
Superior parietal	-0.0075	213.09	-25.1	-58.6	63.1
Cuneus	0.008	135.02	-4.5	-92.9	15.2
Superior parietal	-0.011	223.84	-17.7	-65.5	52.2
Lateral orbito frontal	-0.014	104.28	-15.4	22.2	-17.4
Supramarginal	-0.015	68.64	-57.3	-37.2	34.7
Superior parietal	-0.015	291.27	-23.9	-81.4	22.3
Supramarginal	-0.016	49.85	-37.6	-35.5	35.8
Posterior cingulate	-0.022	100.49	-6.2	-2.2	39.3
Banks of superior	-0.023	30.53	-44.7	-49.9	9.2
Pericalcarine	0.024	88.31	-17.7	-77.5	6.2
Rostral middle frontal	-0.025	69.16	-23.6	55.7	13.1
Postcentral	-0.026	37.82	-44.8	-20	50.8
Medial orbito frontal	-0.03	148.09	-6.4	25.4	-17.4
Fusiform	-0.03	143.69	-40.6	-53.3	-13.5
Rostral middle frontal	-0.03	16.71	-31.1	55.5	-8.6
Paracentral	-0.032	132.83	-11.4	-41	65.9
Inferior parietal	-0.033	10.2	-35	-84.8	16.2
Superior parietal	-0.037	25.96	-16.5	-82.7	33.9
Cuneus	-0.037	40.53	-4.7	-84.2	29.1
Frontal pole	-0.039	15.46	-5.9	60.8	-13.5
Postcentral	-0.04	8.68	-46.6	-17.7	15.5
Lateral orbito frontal	-0.04	9.17	-25.1	25.1	-14.9
Transverse temporal	0.04	13.31	-40.7	-25.8	9

Table 62: Clusters of significant difference in the left hemisphere between individuals with RE and healthy controls. Only clusters greater than 10 mm² and < p=0.05 were included. Significance (p=), Size of cortical area, X, Y and Z MNI coordinates for the vertices in the cluster with the most significance.

Left hemisphere corrected results					
Cortical region	Significance	Size(mm^2)	X	Y	Z
Lateral orbitofrontal	-0.0001	8322.78	-26.3	23.8	-6
Superior frontal	-0.0001	2299	-15.5	45.7	38.6
Isthmus of cingulate	-0.002	1420.54	-15.4	-54.7	8.6
Precentral	-0.044	928.66	-36.8	-18.3	64.5

Table 63: Clusters of significant difference in the left hemisphere between individuals with RE and healthy controls after correction for multiple comparisons. X, Y and Z; MNI coordinates

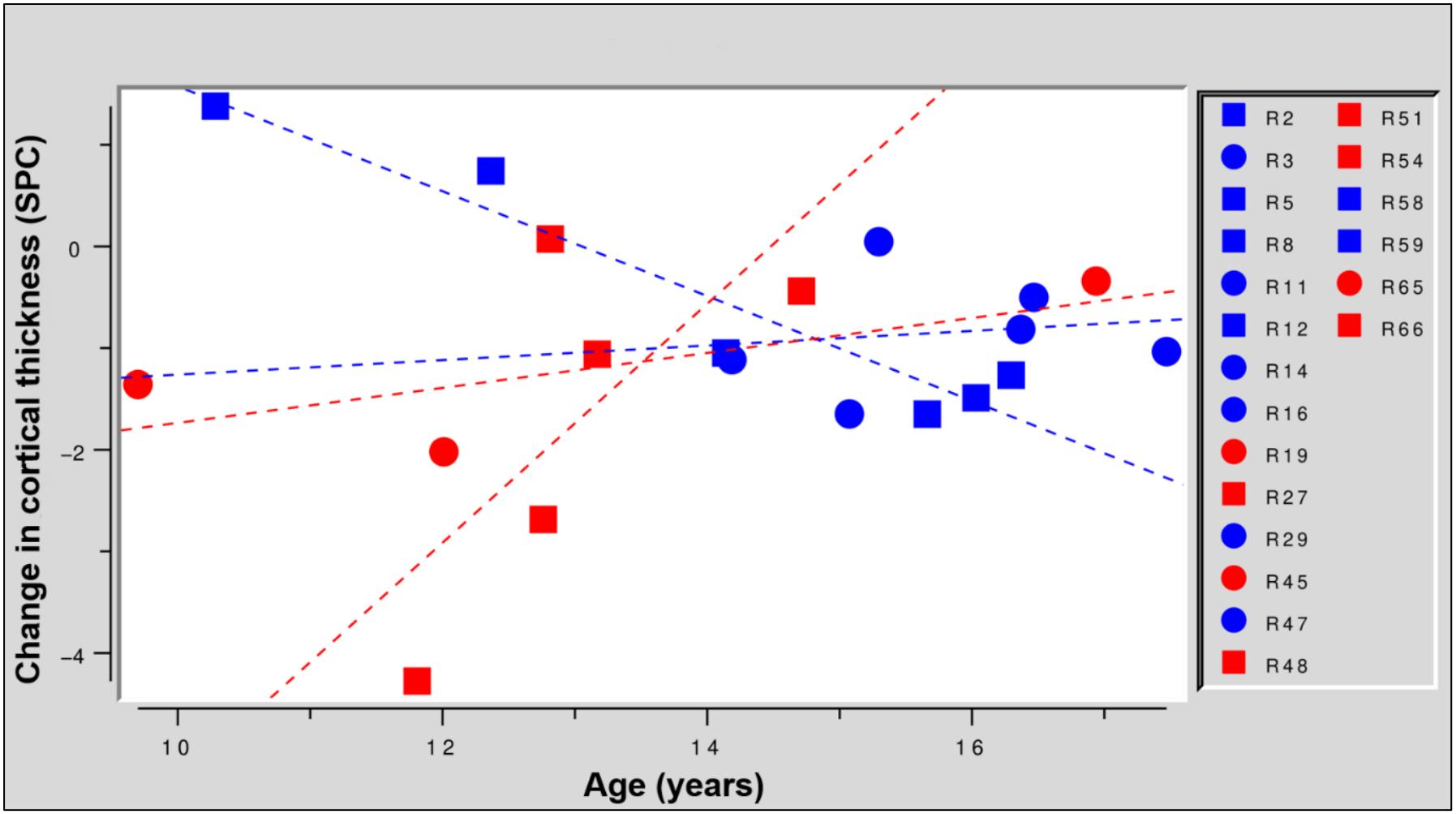


Figure 46: Graph of change in cortical thickness in the left lateral orbital cluster in individuals with RE compared to healthy controls. The right-side panel includes the participant's codes. RE group in red and healthy controls in blue. Squares points are males and circles females. Included are regression lines for sex. Age is calculated as the average age between the two time points. SPC: Symmetrised per cent change. This graph demonstrates an excess of cortical thinning in the left lateral-orbital region in younger individuals with RE, in particular males compared to healthy controls whereas thinning appears to increase in the healthy controls after the age of 14 years. The control males demonstrate cortical thickening under thirteen years of age.

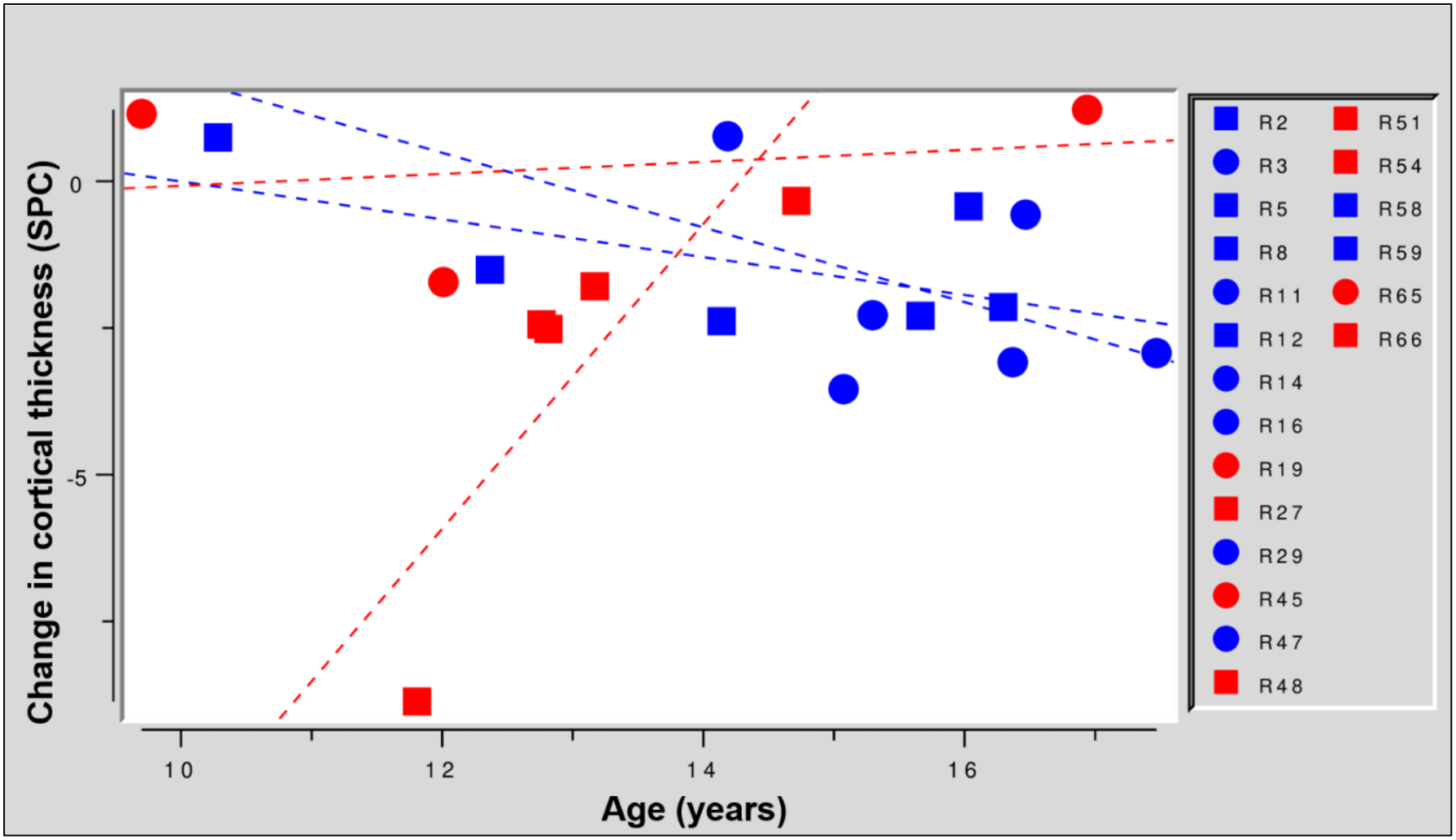


Figure 47: Graph of change in cortical thickness in the left superior frontal cluster in individuals with RE compared to healthy controls. The right-side panel includes the participant's codes. RE group in red and healthy controls in blue. Squares points are males and circles females. Included are regression lines for sex. Age is calculated as the average age between the two time points. SPC: Symmetrised per cent change. This graph demonstrates a reduction in cortical thinning in the RE group compared to healthy controls where there is an increase in cortical thinning with time. There is an outlier in the RE group which is R48.

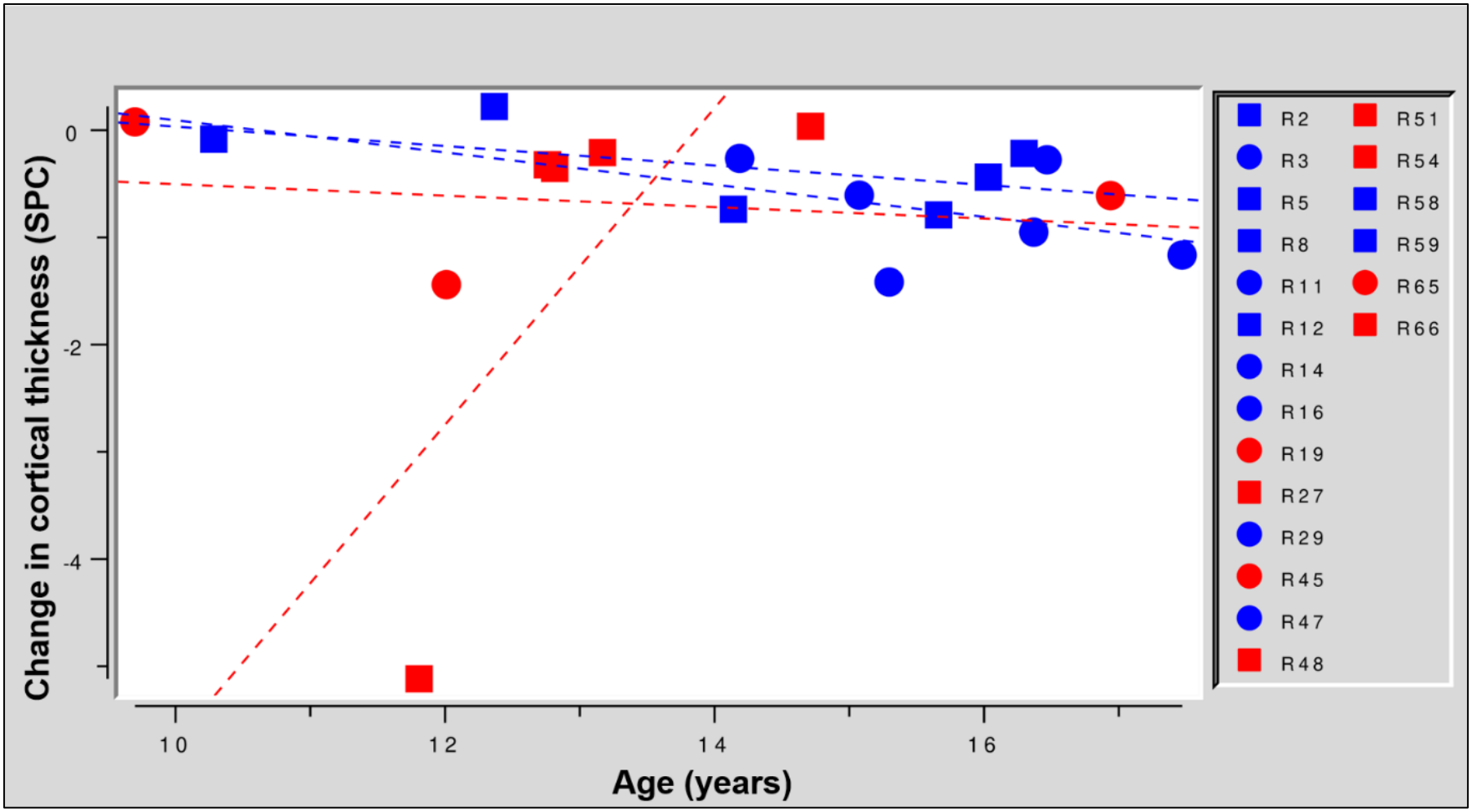


Figure 48: Graph of change in cortical thickness in the left isthmus of the cingulate in individuals with RE compared to healthy controls. The right-side panel includes the participant's codes. RE group in red and healthy controls in blue. Squares points are males and circles females. Included are regression lines for sex. Age is calculated as the average age between the two time points. SPC: Symmetrised per cent change. If the outlier R48 is removed from this graph, then then it would appear that there is little difference in the change in cortical thickness of the isthmus of the cingulate between individuals with RE and healthy controls.

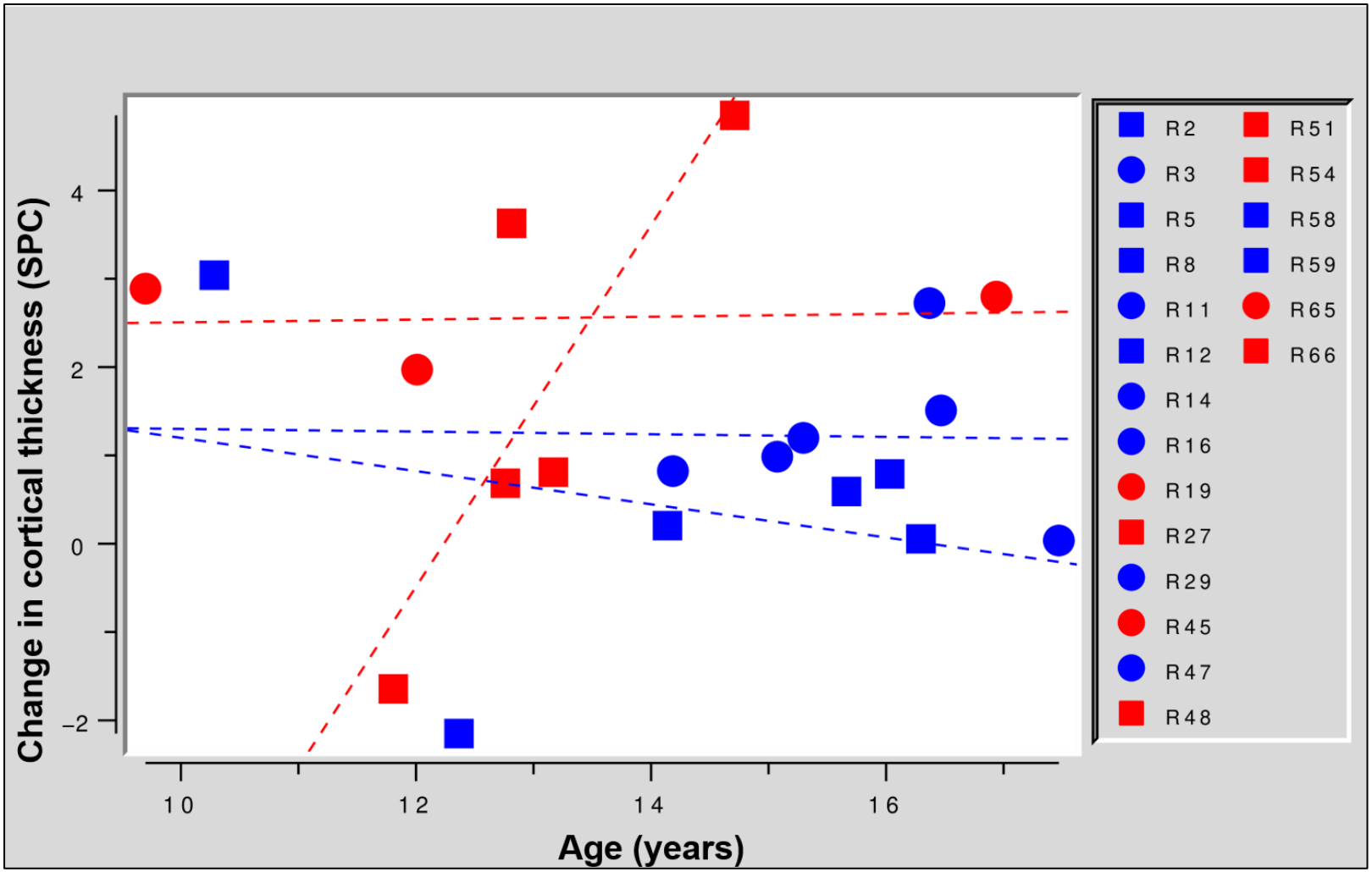


Figure 49: Graph of change in cortical thickness in the left pre-central gyrus cluster in individuals with RE compared to healthy controls. The right-side panel includes the participant's codes. RE group in red and healthy controls in blue. Squares points are males and circles females. Included are regression lines for sex. Age is calculated as the average age between the two time points. SPC: Symmetrised per cent change. This graph demonstrates thickening of the left pre-central gyrus in males with RE with age, whereas in females there is a steady thickening of the cortex in females with RE. In the healthy control group, thickening of this cortical region appears to reduce with age.

The difference in SPC in the left hemisphere is presented in Figure 45, and the data for these uncorrected clusters are in Table 62. The regions with the most significant clusters included; the insula, superior frontal, pre and postcentral gyrus, the precuneus and superior parietal region and the lingual gyrus, these all demonstrated a difference in the change in cortical thickness between the RE and control group with age. Correction for multiple comparisons using Monte-Carlo null-Z simulation reduced the number of clusters for analysis.

After correction, four clusters remained with peak significance in the lateral orbitofrontal, superior frontal, isthmus of the cingulate and pre-central gyri (Table 63). The clusters were quite large, ranging between 928.66-8322.78 mm^2. The reported region names are where the peak significance lies. The lateral orbitofrontal cluster included the anterior portion of the superior temporal, middle temporal and inferior temporal lobes, insular, pars opercularis, and the inferior portion of pars triangularis and orbitalis. The superior frontal region cluster includes parts of the rostral middle and caudal middle frontal gyrus. The isthmus of the cingulate cluster includes the precuneus and lingual gyrus. Finally, the pre-central gyrus cluster incorporated part of the caudal middle frontal regions. The graphical data supports some of the significant clusters.

Three of the significant clusters appear to be supported by the graphical data. This included the left lateral orbital frontal, superior frontal and pre-central gyrus. In the lateral orbital frontal cluster (Figure 46) there is evidence for an excess of cortical thinning in the left lateral-orbital region in younger individuals with RE, in particular, males compared to healthy controls. In the healthy controls after the age of 14 years there is an increase in cortical thinning. Under 13 years of age the control males demonstrate cortical thickening. In the superior frontal cluster, (Figure 47) there was a decrease in cortical thinning with age whereas in the control group there appears to be an increase in cortical thinning with age. In the left pre-central cluster (Figure 49), there is reduction in cortical thinning with age in the RE group compared to controls. This phenomenon leads to cortical thickening and is seen within the male participants of the RE group. In the control group, in females, there is little change in cortical thinning with age whereas in males there is a reduction in cortical thickening with age. Overall, these findings would suggest altered cortical development in the lateral orbitofrontal, superior frontal lobe and pre-central gyrus in individuals with RE.

6.4.4.4 *Right hemisphere longitudinal comparison of changes in cortical thickness between rolandic epilepsy and control group*

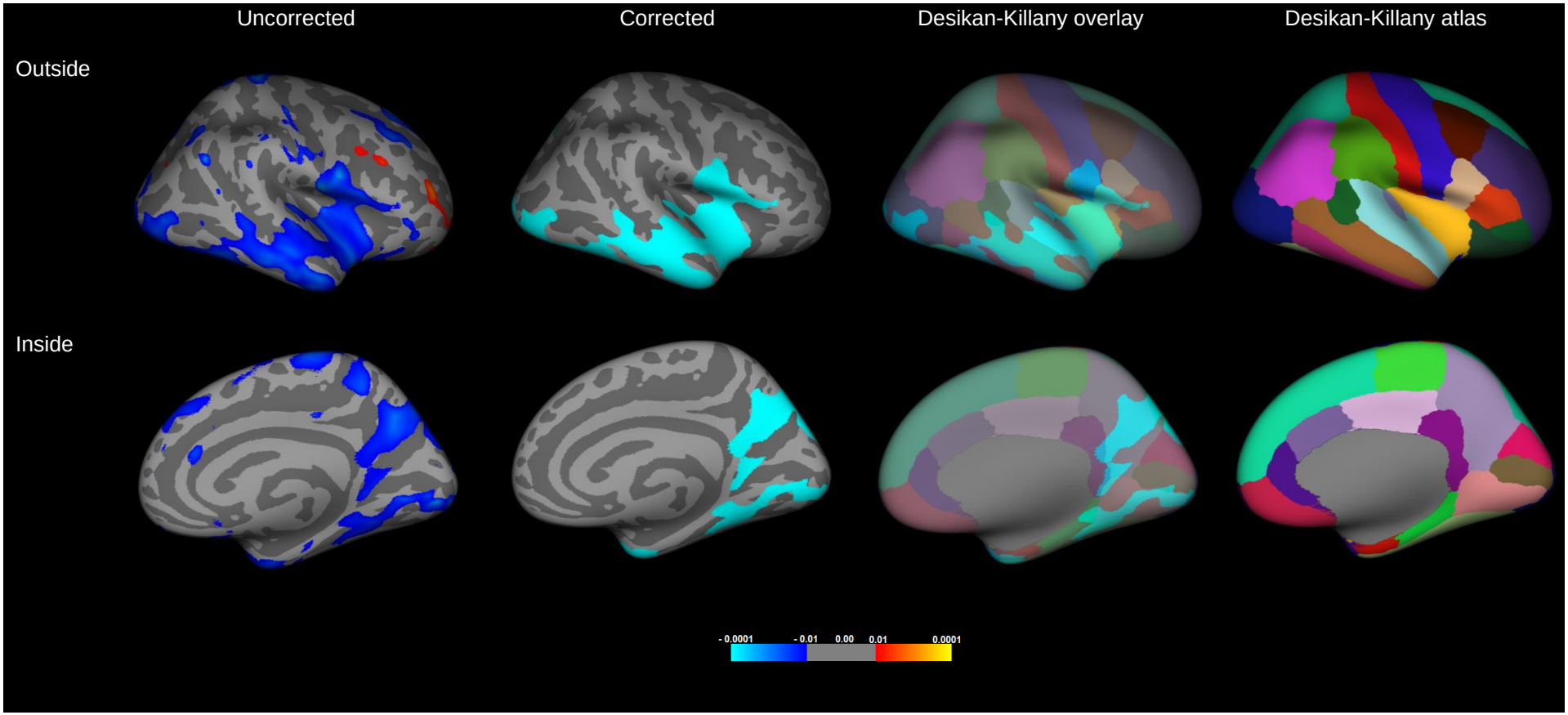


Figure 50: Differences in changes in cortical thickness in the right hemisphere between participants with RE and healthy controls between baseline and follow-up. Top row, outer hemisphere. Lower row inner hemisphere. From left to right: First column uncorrected inflated pial model, second column; inflated pial corrected model, third column; inflated pial with Desikan-Killany overlay and fourth column Desikan-Killany atlas on an inflated pial model. Analysis corrected for age and sex. All results corrected for familywise error using a Monte-Carlo simulation (p=0.05). Clusters colours in the first column reflect the level of significance ranging from p=-0.0001 (blue) to 0.0001 (red). Blue clusters represent a decrease in cortical thinning with age compared to the control group, which increased in cortical thinning with age Cluster colours in the other columns are arbitrary. Degrees of freedom: 12

The results for the right hemisphere are contained in Right hemisphere longitudinal comparison of changes in cortical thickness between rolandic epilepsy and control group

Figure 50 and the data in Table 64.

Right hemisphere					
Cortical region	Significance	Size(mm²)	X	Y	Z
Postcentral	-0.00083	11766.91	53.3	-7.7	11.6
Inferior parietal	-0.0014	94.45	47.2	-59.5	28.8
Postcentral	-0.0016	967.12	13.2	-33.3	71.5
Rostral middle frontal	0.0024	389.01	36.8	49.4	14.4
Lateral orbito frontal	-0.0025	611.77	15.9	15.8	-21.6
Precuneus	-0.0031	2294.69	6	-67.5	32.4
Rostral middle frontal	-0.005	503.62	28.3	32.4	34.3
Post central	-0.005	192.26	42.4	-23	51.1
Posterior cingulate	-0.006	25.31	6.5	-27.6	40.2
Precuneus	-0.0063	549.35	11	-48.4	53.8
Superior frontal	-0.0074	817.26	20.9	30.1	46.9
Inferior parietal	-0.0075	238.56	34.1	-63.5	46
Caudal anterior	-0.0088	80.69	5.9	31	19.7
Post central	-0.012	281.94	55.9	-10.1	28.7
Rostral middle frontal	0.012	70.43	40.8	21.2	28.3
Pars triangularis	-0.014	185.98	50.4	33.3	-6
supramarginal	-0.015	29.93	60.9	-40	25.2
fusiform	-0.016	49.89	37.5	-15.8	-29.9
supramarginal	-0.017	34.75	55.6	-31.6	45.2
Lateral orbitofrontal	-0.023	32.52	12.1	50.9	-21.2
Inferior parietal	-0.024	14.1	50.7	-55.3	13.9
Lateral occipital	-0.026	176.34	25.6	-87.1	16.4
Lateral occipital	-0.028	34.53	10.6	-95.2	13.8
Lateral orbitofrontal	0.028	19.31	20.8	49	-12
Caudal middle frontal	0.032	54.51	32.7	7.8	31
Para-hippocampal	-0.035	46.37	23.2	-19.6	-26.5
supramarginal	-0.036	10.1	53.1	-35.2	47
Superior frontal	-0.04	114.62	14.6	0.2	65.7
Inferior parietal	0.04	11.76	33.9	-74.8	19.4
Superior frontal	-0.044	44.25	9	9.9	55
Medial orbitofrontal	-0.044	10.9	7	20.3	-12.3
Middle temporal	-0.046	10.45	47.6	-58.5	0.6

Table 64: Clusters of significant difference in the right hemisphere between individuals with RE and healthy controls. Only clusters greater than 10 mm² and < p=0.05 were included.

Right hemisphere					
Cortical region	Significance	Size(mm²)	X	Y	Z
Lateral occipital	-0.0001	11766.91	21.3	-98.7	5.3
Precuneus	-0.0001	2294.69	8.5	-53.1	20.4

Table 65: Clusters of significant difference in the right hemisphere between individuals with RE and healthy controls after correction for multiple comparisons. Included are significance (p=), size of cortical area, X, Y and Z, MNI coordinates of the part of the cluster with the highest significance.

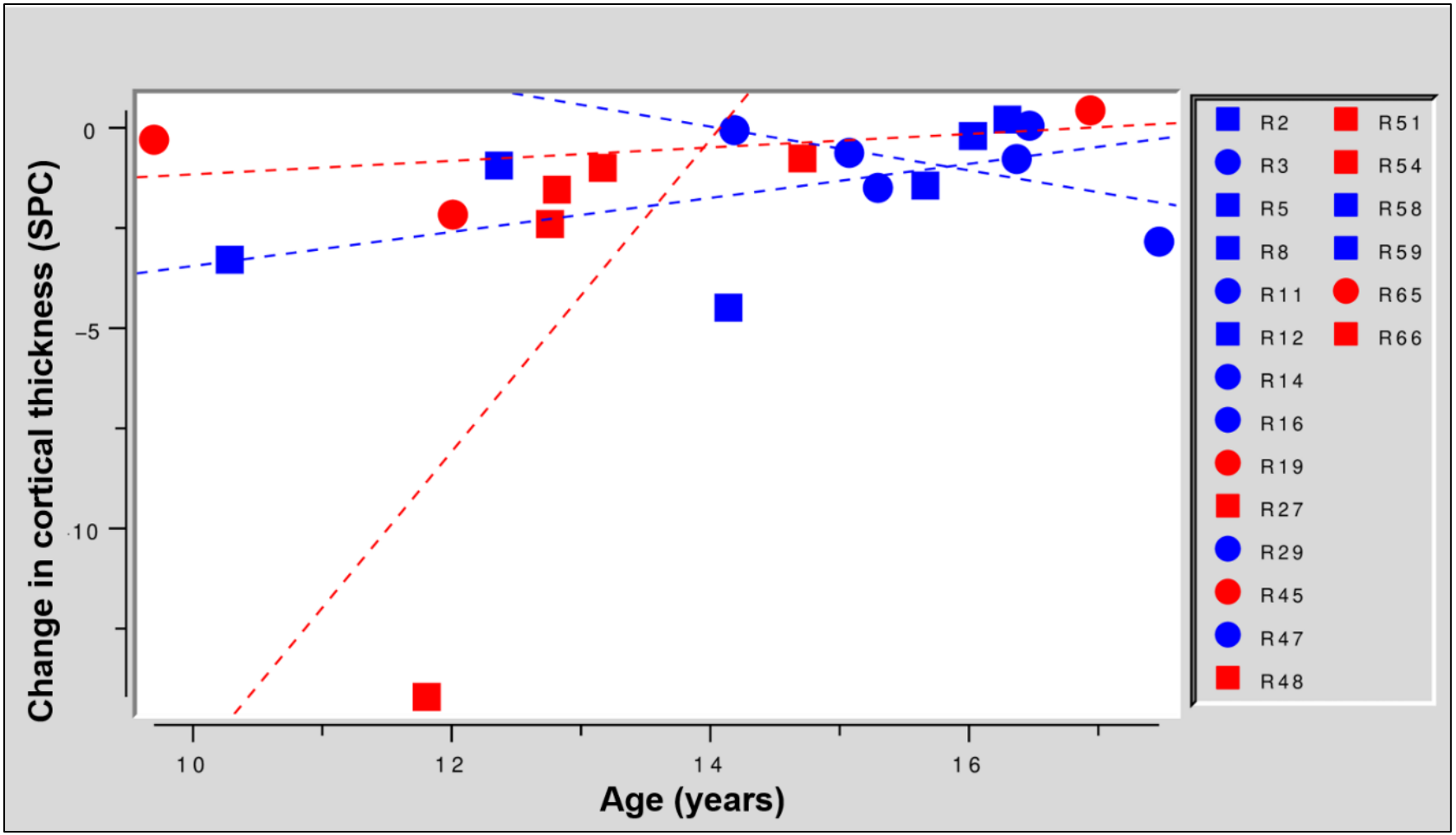


Figure 51: Graph of change in cortical thickness in the right lateral occipital cluster in individuals with RE compared to healthy controls. The right-side panel includes the participant'ss codes. RE group in red and healthy controls in blue. Squares points are males and circles females. Included are regression lines for sex. Age is calculated as the average age between the two time points. SPC: Symmetrised per cent change. This graph demonstrates cortical thinning in the right lateral occipital cluster in both individuals with RE and healthy controls. There is an outlier in the RE group, R48. If this outlier was removed it is unlikely that there would be a significant difference between the groups.

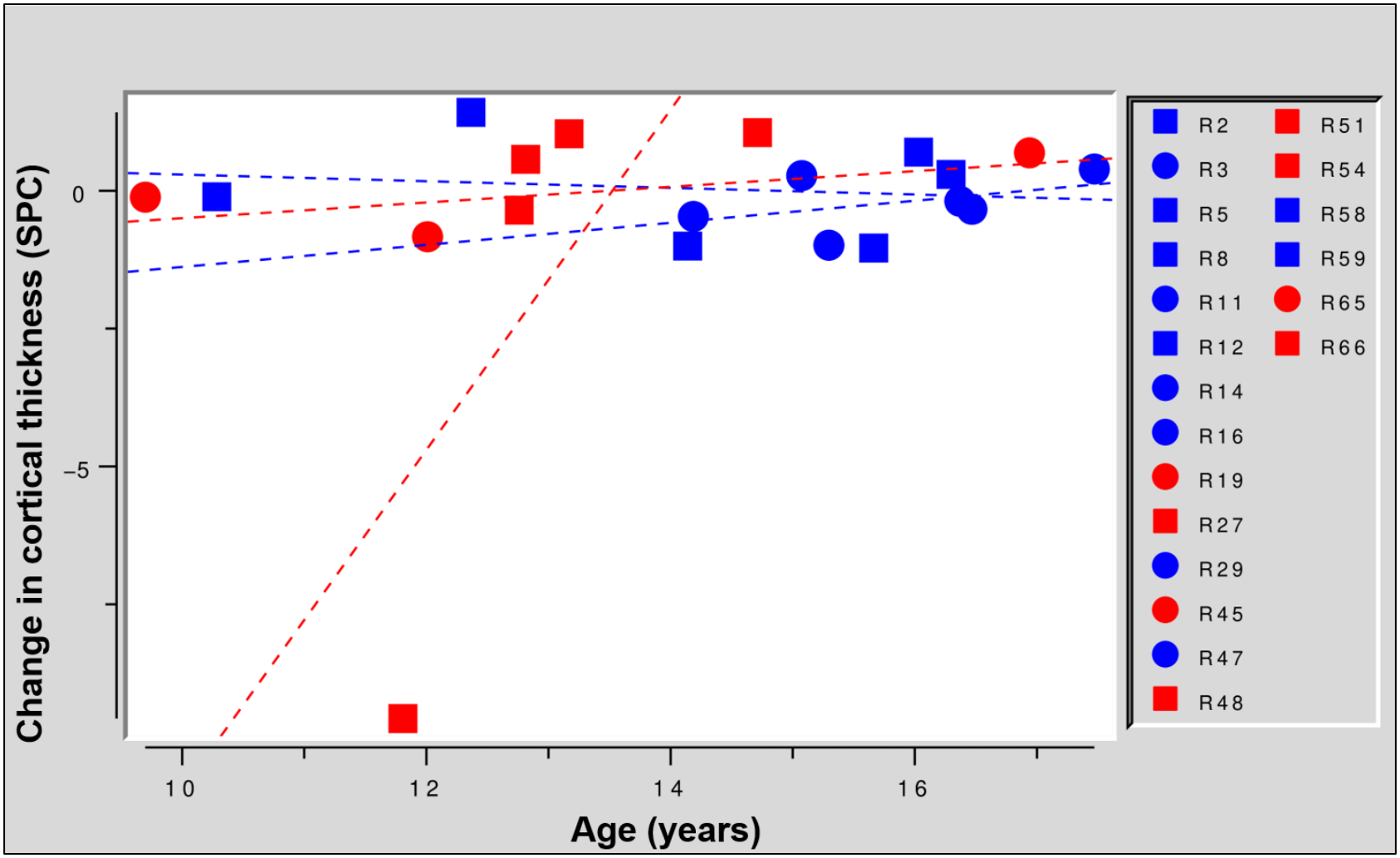


Figure 52: Graph of change in cortical thickness in the right precuneus cluster in individuals with RE compared to healthy controls. The right-side panel includes the participant's codes. RE group in red and healthy controls in blue. Squares points are males and circles females. Included are regression lines for sex. Age is calculated as the average age between the two time points. SPC: Symmetrised per cent change. This graph demonstrates a similar mixture of thinning and thickening the RE and healthy control

group. As seen in the previous graph there is an outlier in the RE group, R48. If this outlier was removed it is unlikely that there would be a significant difference between the groups.

In the right hemisphere, there were eight cortical regions which contained highly significant uncorrected clusters (Table 64) representing a difference in the change of the cortical thickness with age between individuals with RE and healthy controls. The clusters were found within the central, inferior parietal and precuneus, superior and lateral orbitofrontal and the posterior and caudal anterior cingulate regions. With correction using Monte-Carlo null Z simulation, only two clusters survived.

After correction, significant clusters had peak significance over the post-central gyrus and precuneus (Table 65). The lateral occipital gyrus cluster spans from the frontal lobe to the occipital lobe via the temporal lobe. In involves portions of the pre-central gyrus with spread into the pars opercularis and triangularis. A large proportion of the insula and middle temporal gyrus with lesser involvement of the superior and inferior temporal lobes with spread into the lingual gyrus and lateral occipital lobe. The precuneus cluster is seen within the isthmus of the cingulate and borders into part of the cuneus and superior parietal region. Analysis of the graphical output would suggest that their significant differences may have been influenced by an outlier.

Figures 49 and 50 would suggest there is little difference between the change in cortical thickness between the RE group and a healthy control group. These graphs implicate a male participant in the RE group who presents as an outlier in the dataset. Overall, these results would suggest that there is no longitudinal evidence for altered cortical development in the right hemisphere of individuals with RE.

6.4.5 **Longitudinal subcortical changes**

6.4.5.1 *Intra-cranial volume and its constituents*

To calculate the change in subcortical volume between the time-points, the values were converted into a rate of change in cortical thickness ($mm^3/year$) and SPC. Presented below are the changes in the volume of ICV, white matter and grey matter. Significant differences were seen in changes in ICV (SPC p = 0.014) and WM (SPC p = 0.049). In adolescents with RE, ICV and WM were seen to increase, which is the reverse of volume changes seen in healthy controls. Interestingly there, was a similar decrease in the grey matter between the adolescents with RE and healthy controls.

A MANOVA model with sex and average age between baseline and follow-up time points as covariates was used to analyse measures of SPC and rate. The model was significant between groups for SPC (p=0.024) and rate (p= 0.022). In the SPC corrected model found ICV (p=0.014) and WM (p=0.049) to be significantly different between groups. Post-hoc ANOVA revealed that the covariate sex was significant for ICV (p=0.048), and the covariate average age was significant for ICV (p=0.021) with WM approaching significance (p=0.063). The corrected model for rate produced similar significant findings as SPC analysis except for WM did not achieve significance.

	RE n = 8		Control n = 12		DF	Rate Stats (p=)	SPC Stats (p=)	SPC effect size (d)
	Rate (mm^3/year)	SPC (%)	Rate (mm^3/year)	SPC (%)				
Change in ICV	908.41 ± 20043.12	0.09 ± 1.30	-245.34 ± 11512.9	-0.03 ± 0.73	17	**0.013**	**0.014**	0.11
Change GM	-10378.37 ± 8002.22	-1.35 ± 1.02	-12295.01 ± 7849.02	-1.67 ± 1.06	17	0.575	0.555	0.3
Change in WM	2953.48 ± 5094.49	0.65 ± 1.13	-763.19 ± 2049.56	-0.18 ± 0.48	17	0.052	**0.049**	**0.96**

Table 66: Changes in ICV, GM and WM volumes in participants with Rolandic epilepsy and controls. Included are measures of rate in mm^3/year and symmetrised per cent change. Stats: MANOVA with the covariates sex and age. DF = Degrees of freedom. Effect sizes for SPC (symmetrised per cent change) (Cohens d).

6.4.5.2 *Volume of subcortical structures*

	RE n = 8		Control n = 12		DF	Rate stats (p=)	SPC stats (p=)	SPC effect size (d)
	Rate (mm³/year)	SPC (%)	Rate (mm³/year)	SPC (%)				
Left thalamus	19.81 ± 125.72	0.21 ± 1.64	-57.79 ± 110.07	-0.77 ± 1.51	17	0.374	0.410	0.62
Right thalamus	-17.30 ± 97.14	-0.22 ± 1.38	-70.56 ± 54.59	-1.02 ± 0.80	17	0.230	0.211	0.71
Left putamen	78.96 ± 141.30	1.58 ± 2.70	-17.32 ± 59.11	-0.34 ± 1.11	17	0.053	**0.046**	**0.93**
Right putamen	43.14 ± 99.73	0.78 ± 1.87	-51.82 ± 38.05	-0.93 ± 0.73	17	**0.038**	0.051	**1.20**
Left caudate	-12.50 ± 41.82	-0.34 ± 1.14	-40.79 ± 29.80	-1.06 ± 0.79	17	0.338	0.371	0.73
Right caudate	-6.41 ± 42.52	-0.18 ± 1.05	-37.30 ± 37.49	-0.93 ± 0.93	17	0.069	0.066	0.76

Table 67: Changes in volume of subcortical structures in participants with RE and controls.

A decrease in volume: Blue, Increase in volume: Orange. Included are measures of rate in mm³/year and symmetrised per cent change (SPC). Stats: MANOVA with age and sex as covariates. DF: Degrees of freedom. Included are effect sizes for SPC (Cohens d).

Symmetrised per cent change and rate of change were calculated for the bilateral, thalamus, putamen and caudate (Table 67). Most of the subcortical structures decreased in volume with age, and this appears at the greatest rate in the healthy control group. In adolescents with RE, an increase in the volume of the left thalamus and bilateral putamen was recorded. A MANOVA model with the average age at baseline and follow-up and sex as covariates found no significant differences between the groups (p=0.236). In the corrected model, the left putamen was significant (p =0.046), and the right putamen (p=0.051) and right caudate (p=0.066) approached significance. Post-hoc ANOVAs found that there was an approach toward significance for sex in the rate of change for the left putamen (p=0.057) and the average age was significant for the right caudate (p=0.040). Interestingly, when the effect of group was investigated the right putamen (p=0.013), caudate (p=0.016) and left putamen (p=0.033) were significant with the right thalamus (p=0.051) and left caudate (p=0.088) trending towards significance. Similar findings were obtained for the analysis of rate except for the right putamen was significant in the corrected MANOVA model.

6.5 Conclusions

This chapter has produced many interesting results, and it has revealed the power of a longitudinal neuroimaging study. The following section is divided into cross-sectional and longitudinal findings.

6.5.1 Cross-sectional

6.5.1.1 *Exploratory analysis found regions with thicker cortex in individuals with RE.*

Despite the findings seen in vertices analysis, this does not translate into direct comparisons of cortical thickness across different cortical parcellations. In both the baseline and follow-up cohorts, there was a region of significantly increased thickness in individuals with RE compared to healthy controls. In the baseline cohort this was the right medial orbital frontal gyrus (p= 0.037, d=0.49) and in the follow-up cohort the left rostral middle frontal region (p=0.002, d= 0.31). There is a possibility that these two significant features are due to chance or type 1 error as the regions are few and the number of multiple comparisons were twenty-six per hemisphere. Nevertheless, if these features are not due to chance or type 1 error, then this is evidence of altered development in the frontal lobes of individuals with RE. This feature has been seen in other studies.

There is evidence in the literature to support this finding of altered cortical thickness in the frontal lobe of children with RE. The evidence includes regions of increased thickness in the left rostral middle frontal region (Garcia-Ramos *et al.*, 2015) and thickened cortex in the bilateral middle and inferior frontal regions bilaterally (Pardoe *et al.*, 2013). Nevertheless, the studies contained no measures of cortical thickness, they only presented statistical difference, so it is hard to make direct comparisons. Despite this, the similarity between the studies for areas of thicker cortex in individuals with RE could be interpreted as a reduction in cortical thinning.

6.5.1.2 *Direct comparisons of cortical thickness and thinning correlations help understand cortical development in individuals with RE*

The use of simple contrast between participants with RE and healthy controls in the baseline cohorts revealed large clusters of predominantly thinner cortex in the left hemisphere which were at their most significant in the frontal lobe and superior temporal lobe. Whereas in the right hemisphere there was no over-arching theme with one thick cortical region in the medial orbitofrontal regions and another thin in the inferior parietal lobe. The lack of a theme in the right hemisphere is seen in the follow-up cohort.

In the right hemisphere, no highly significant clusters recorded in the baseline cohort were seen in the follow-up cohort. In the right hemisphere, predominantly clusters of thicker cortex were seen in the insula, inferior temporal and isthmus of the cingulate was seen whereas there is a cluster of thinner cortex in the pre-central gyrus. Conversely, in the left hemisphere clusters seen in the baseline cohort were seen in the follow-up cohort, this included clusters in the superior and caudal middle frontal and superior temporal regions. In the follow-up cohort, the frontal regions were thicker than controls, which were in marked contrast from the baseline cohort, whereas the superior temporal cluster was thinner than controls in both cohorts. The frontal regions were implicated in the exploratory parcellation analysis. In the baseline cohort, the right medial orbital frontal lobe was thicker than controls, and in the follow-up cohort, the left rostral mid-frontal region was thicker. There are some findings which have similarities with literature.

Overliet *et al.* found clusters of thinner cortex in the superior temporal sulcus (Geke M. Overvliet, Besseling, Jansen, *et al.*, 2013) whereas in the right no difference was detected. Garcia-Ramos *et al.* found thinner cortex in the left rostral middle frontal ($p < 0.005$) and inferior temporal gyrus (Garcia-Ramos *et al.*, 2015) but no similar features in the right hemisphere. In the Kim study, within the left hemisphere, patches of thicker cortex were reported in the superior temporal gyrus. Not reported in the text but apparent in the figures were significant clusters of thicker cortex in the left frontal lobe. There were no similarities in the right hemisphere. (Kim et al., 2015).

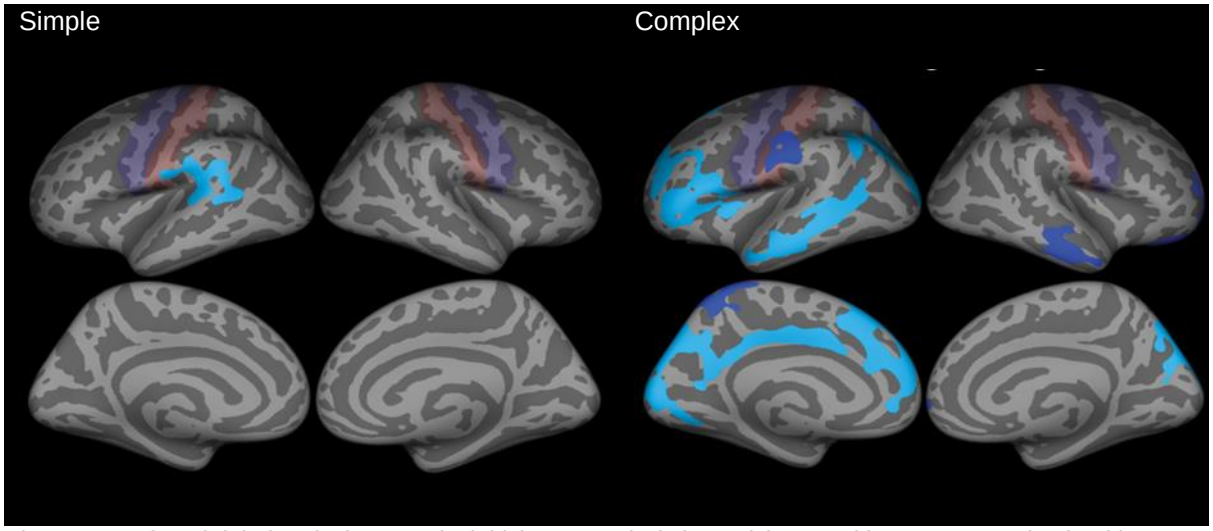


Figure 53: Left and right hemisphere cortical thickness analysis in participants with RE compared to healthy controls. Left: Simple contrast corrected for multiple comparisons. Right: Complex contrast corrected for age and sex. Lateral aspect: Top. Medial aspect: Pre (purple) and post-central gyrus (red) Within the literature, this study has the most power due to a sample size of 24 participants with RE and 24 healthy controls. Extracted from Overvliet et al. (2013).

Regarding brain imaging in seizure remission, Pardoe *et al.* report on cortical thickness in individuals with RE aged 16, which would be comparable to this study's follow-up cohort. They found clusters of thicker cortex in the inferior and middle frontal gyri. In the right hemisphere, there was thinner cortex in the right central sulcus.

Regarding the correlation between age and cortical thickness, the term apparent thinning or thickening will be used. To recap in the baseline cohort, in the left hemisphere, apparent thinning was seen in clusters within the frontal and temporal lobes with apparent thickening in the posterior cingulate and bordering cuneus. In the follow-up cohort, this had changed to clusters of apparent thickening predominantly in the frontal and parietal regions with small clusters in the temporal and occipital lobe. The cingulate and cuneus show no differences with controls. Similarly, apparent thinning was seen in the right hemisphere in the baseline cohort.

In the right hemisphere, in the baseline cohort, the clusters of apparent thinning were predominantly seen in the frontal regions and thickening in the parietal regions. Whereas in the follow-up cohort thickening predominated, but it was seen in every lobe. The only clusters of apparent thinning were seen in the frontal lobe. Note that these regions are similar or are close to regions of altered thickness

identified in the simple analysis. Increased apparent thinning in active epilepsy has been reported in the literature.

Overliet *et al.* found in active epilepsy increased apparent thinning in the left hemisphere, within the superior and middle frontal regions and the middle temporal gyrus and in the right the rostral middle frontal, lateral orbitofrontal cortex and superior parietal cortex. Garcia-Ramos also found apparent clusters of thinning in the bilateral middle frontal gyrus (p=<0.005) (Garcia-Ramos *et al.*, 2015).

6.5.1.3 *The right hemisphere has a large area of apparent cortical thickening in seizure remission*

The only region to survive multiple comparisons was seen in the right hemisphere this was a large area of apparent thickening with age which incorporated parts of the pre-central, supramarginal gyrus, inferior parietal, banks of the superior temporal sulcus and superior temporal gyrus. Pardoe *et al.* found this phenomenon in a group of individuals with RE mean age 16 years ~ 9 years after the onset of epilepsy (Figure 54). They found increased cortical thickness within the post-central, supramarginal gyrus and superior temporal gyrus. This finding would suggest that altered cortical thickness could be apparent in seizure remission.

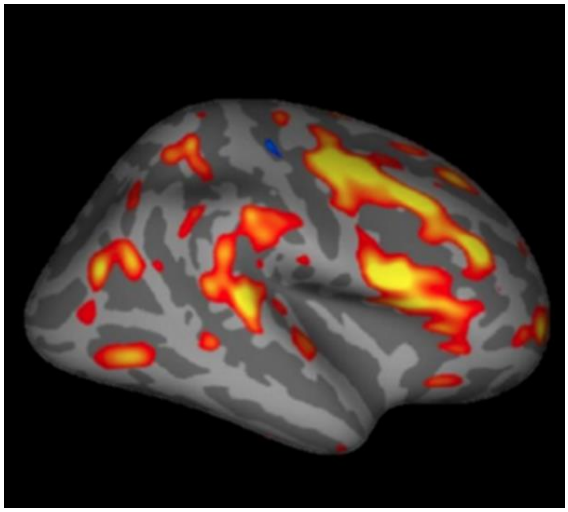


Figure 54: Increased cortical thickness in the right hemisphere in individuals with RE in possible seizure remission. Extracted from Pardoe et al (2013)

This reproducible finding could represent the emergence of part of the epileptic network or is a region which has been affected by either spikes or seizure generation. It may be that the seizures or spikes halt the thinning of the cortex over these regions, or it may be that the restricted thinning is a precursor of spikes and seizures. Nonetheless, this feature was only apparent in seizure remission, so it would most likely implicate an increase in cortical thickness in seizure remission. This would imply that there is either a disruption of either grey or white matter development. There is limited evidence on how other epilepsies have changes in cortical structure with seizure remission.

Some good controlled evidence comes from a longitudinal study of idiopathic temporal lobe epilepsy. There is evidence of diffuse grey matter atrophy in patients with temporal lobe epilepsy (TLE) without hippocampal sclerosis (HS) in seizure remission. This included atrophy in the fusiform gyrus, hypothalamus, rolandic operculum, insula, pre- and postcentral gyrus, inferior parietal and temporal lobes ipsilateral to EZ, as well as in contralateral cerebellum and middle temporal gyrus (Alvim *et al.*, 2016). Furthermore, in a controlled cross-sectional study of individuals with childhood absence epilepsy five decades into seizure remission there was an area of thicker cortex which spanned between the postcentral gyrus and superior parietal lobe (Garcia-Ramos *et al.*, 2017). These findings

would suggest that aberrant development of brain structure could be persistent or become present after seizures have remitted.

6.5.1.4 *Intra-cranial volume is large in some children with Rolandic epilepsy*

An overview of cranial volumes was performed in the baseline and follow-up cohorts. In the RE groups at baseline and at follow-up, there was increased intracranial volume (ICV), this was seen despite no significant difference in total GM, WM or GM/WM ratio. This apparent enlargement may have been due small sample size and caution is required in the interpretation; however other studies have found similar results. Lin *et al.* in 13 participants with RE, found a significantly larger brain volume between individuals with RE and control with an effect size of d= 0.95. Similarly, Kim *et al.* produced supplementary data detailing total brain volume in 20 participants with RE. Despite being non-significant compared to controls, a moderate effect size of d = 0.46 was calculated (Kim *et al.*, 2015). Enlarged ICV can be reported in some neurodevelopmental disorders.

ICV is a measure of the volume of the cranial cavity as outlined by the supra-tentorial dura matter (Eritaia *et al.*, 2000). An enlarged ICV would be evidence of a neuro-development disorder. Large ICVs are seen in children with autism, and this can be evidence of brain overgrowth (Courchesne, Carper and Akshoomoff, 2003). It is important to note that increased ICV in autism is apparent in the first year of life. It is unclear from these cross-sectional results when the increased ICV is first apparent; however, the difference in ICV has greater significance in the follow-up cohort. If ICV in individuals with RE is apparent from birth, then this would be evidence of altered brain development preceding the generation of seizures. Another different volume was the non-grey or white matter.

Non-grey or white matter volume was significantly different in the baseline and follow-up cohorts. None, GM/WM volume, is influenced by the volume of the cerebral spinal fluid ventricles and blood vessels. The ventricles are the largest component of this volume; therefore, it is suspected that they are influencing the increased none GM/WM volume. In the literature, enlarged ventricles have been reported in RE (Gelisse *et al.*, 2003; Boxerman *et al.*, 2007). Enlarged ventricles, in particular, the lateral ventricles, can be an indicator of a neurodevelopmental disorder. It is said to indicate abnormal prenatal brain development (Lyall *et al.*, 2012) and can be seen in neurodevelopmental disorders such as attention deficit hyperactivity disorder (Wang *et al.*, 2007), and autism spectrum disorder (Turner, Greenspan and van Erp, 2016).

6.5.1.5 *Cross-sectional evidence of altered subcortical volumes in participants with RE was weak.*

There was no statistical significance between children with RE and healthy controls in any of the hypothesised subcortical volumes at baseline or follow-up. The findings of this study are incongruent with the findings of multiple studies (Lin *et al.*, 2012; C. Luo *et al.*, 2015; Garcia-Ramos *et al.*, 2015; Kim *et al.*, 2015). A possible reason for this could be, that in this study, participants were recruited a long time after diagnosis compared to the studies in the literature (Table 39), in those studies recruitment was within 6-13 months of their first seizure.

6.5.2 **Longitudinal**

6.5.2.1 *Parcellation analysis found that cortical development is predominantly altered in the left hemisphere in Rolandic epilepsy*

Exploratory analysis of cortical parcellations found two regions in the left hemisphere with altered cortical development compared to controls. These regions were the caudal anterior cingulate and caudal middle frontal region. The caudal anterior cingulate had evidence of a slight thickening between time points, whereas the caudal middle frontal demonstrated a larger reduction in cortical thickness compared to controls. There is only one longitudinal study of cortical thickness in RE (Garcia-Ramos *et al.*, 2015), and therefore, it is difficult to obtain an insight with these findings. Nevertheless, it is interesting to note that these two regions are located anteriorly and that they border each other. The slight increase in cortical thickness in the caudal anterior cingulate could be a representation of delayed cortical thinning or a loss of white matter integrity whereas, increased cortical thinning in the caudal middle frontal region could be suggestive of increased cortical pruning or an improvement in cortical myelination. These regions are evidence that two different aberrant developmental processes could be apparent in individuals with RE.

6.5.2.2 *The area of change in cortical thickness is restricted in Rolandic epilepsy*

Prospective thinning was measured in each of the groups. In the control group, cortical thinning is widespread in both hemispheres with greater thinning occurring in the right lateral cortical regions compared to those in the left hemisphere. Similar findings were seen in the Garcia-Ramos study within the control group (Garcia-Ramos *et al.*, 2015). There is also evidence from outside the RE literature that adolescents have a decrease in cortical thickness with increased aged predominantly in the frontal lobes (Tamnes *et al.*, 2017).

In contrast, in this study, the RE group had patchy thinning of the cortex in both hemispheres, and there is no clear difference in the amount of thinning between the hemispheres. Patchy thinning was not seen in Garcia-Ramos study where only small regions of thinning of the left isthmus of the cingulate and thickening of the superior part of the pre-central gyrus were seen (Garcia-Ramos *et al.*, 2015). A possible reason for this is that the Garcia-Ramos study followed up their participants two years after onset. Nevertheless, this finding is in keeping with the results of this study, as it would suggest the area of changes in cortical thickness in individuals with RE is reduced. These findings intimate a regional delay in cortical development compared to healthy participants it would suggest either a patchy delay to cortical pruning or a delay in white matter maturation.

6.5.2.3 *Evidence of large reductions in cortical thickness in the left hemisphere.*

Longitudinal vertices cluster analysis revealed regions of altered development in both the left hemisphere. This included clusters with peak significance in the left lateral orbital frontal, superior frontal and precentral regions. These regions demonstrated a positive correlation between the change in cortical thickness and age. In other words, the younger participants in the RE group had the greatest reductions in cortical thickness compared to healthy controls. Similar regions have been identified in the literature; in the Garcia-Ramos study, there were some regions of overlap. These were within the left pars orbitalis, triangularis and opercularis, part of the left rostral middle frontal and superior frontal gyrus (Garcia-Ramos *et al.*, 2015). Similar regions were identified; however, what they represented were different from the findings of this study. These differences may be due to when the participants were recruited.

The Garcia-Ramos study recruited participants soon after diagnosis, whereas in this study, there was a delay in recruitment combined with a prolonged period of follow-up. These two factors could indicate a bi-modal function in cortical development in RE. The Garcia-Ramos, study found over two years reduced cortical thinning around the onset of the epilepsy, whereas this study which started later into the epilepsy and ending in seizure remission demonstrated large decreases in cortical thickness.

These findings would propose that these regions may play a role in the generation of the epilepsy as their thinning is associated with remission of seizures.

6.5.2.4 *ICV and white matter volumes increase in participants with RE in seizure remission.*

An unexpected finding was the increase in the volume of both the intracranial volume and white matter volume in participants with RE. In the healthy control group, the rates of change revealed a decrease in volume. It important to note that large standard deviations in this data would suggest a large amount of heterogeneity within the data; nevertheless, the same degree of variance was seen in the healthy controls group. Despite the problem with variance mean rates were significantly different.

In the RE group, there was an increase in ICV, and this appears to accommodate the increase in white matter volume as the grey matter volume was seen to decrease. In the healthy controls, ICV, GM and WM all demonstrated a reduction in the volume. There have been no studies which have analysed the changes of these volumes in RE in seizure remission. Therefore, it is difficult to know what the change in WM volume and ICV means. Furthermore, due to the small sample size, caution is required in the interpretation of the result. Nevertheless, the increase in ICV could be part of a normal developmental process as a large proportion of the RE group are male, and research indicates that males have a larger increase in white matter between the ages of 4 and 22 (Giedd *et al.*, 1999). Conversely, other longitudinal studies have revealed more nuanced developmental trajectories of ICV and WM development.

In a study by Mills et al., 391 individuals were scanned across four different sites. In the largest dataset, Braintime, which consisted of 209 individuals, they found that ICV increased in volume to around the age of 14 and then decreased after this time, in tandem WM volume increases and then begins to decrease at around the same age (Mills *et al.*, 2016)(Figure 55). As the RE group was scanned around this time, this could be the reason why the white matter and ICV both increased in this group compared to the controls. Nevertheless, if the increases in volume are part of the normal development process, why is there extra growth in an ICV which was enlarged at baseline. Finally, it

may be that the growth in white matter volume along with an increased ICV is another factor in the development of seizure remission as seizures are said to be rare after the age of 15 years (Wirrell, 1998), which occurs at a similar time point to the growth of ICV and white matter volumes.

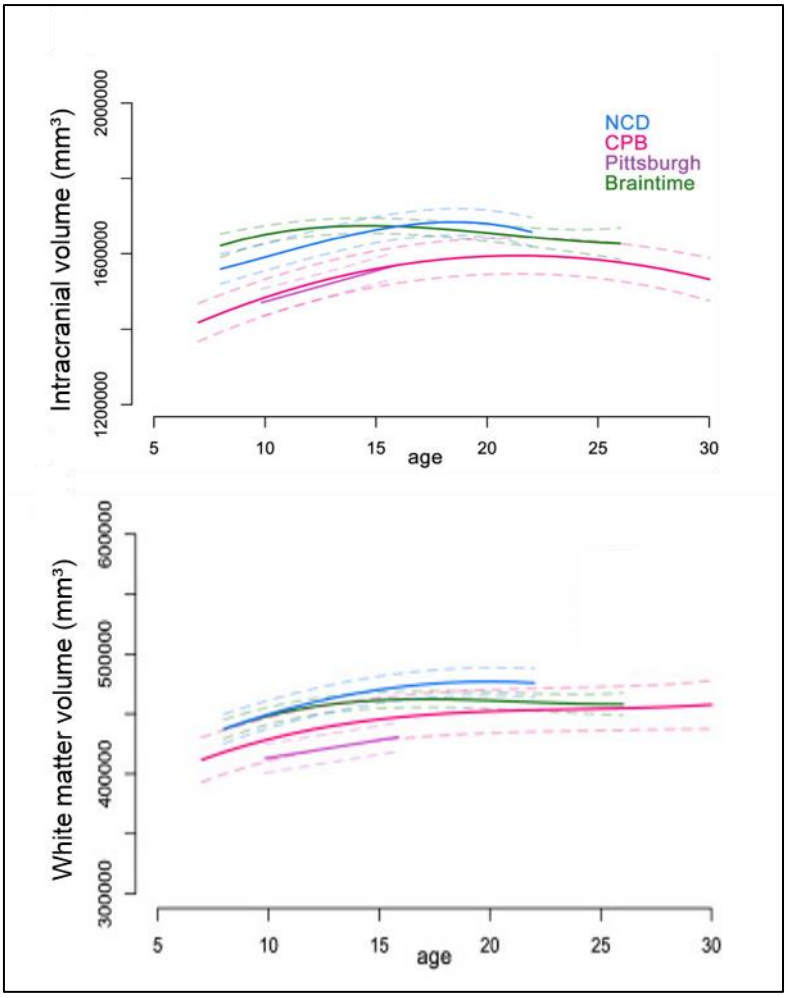


Figure 55: Longitudinal changes in intra-cranial volume and white matter volume in healthy children. Upper graph: Intracranial volume (ICV) in mm³. Lower graph: White matter (WM) in mm³. Four studies are included the study with the greatest power is the Braintime study (green). Extracted and adapted from Mills et al (2016).

6.5.2.5 *Significant increase in the volume of subcortical structures in participants with Rolandic epilepsy in seizure remission.*

Two structures, the bilateral putamen had significantly different development in individuals compared to control. Like the ICV, GM and WM volumes there was a large variance which would suggest either a lack of normal distribution or a heterogeneous group. Nevertheless, there are four features which make these findings more reliable. One, the control group has a similar variance; two, the trend in the controls group is for a decrease in volume, three, the other subcortical structures in the RE group decreased in volume. Finally, the percentage increases in the structures are large, and this is reflected in the large effect sizes. Similar findings were seen in the Garcia-Ramos longitudinal study.

The Garcia-Ramos study found that putamen volume in participants with RE over two years increased in size. The increase in size was very small and could be interpreted as no change; however, the control group had a reduction in the bilateral putamen (Garcia-Ramos *et al.*, 2015) (Table 68). These findings would suggest in individuals with RE that there is either no change or an increase in the volume of the bilateral putamen over time. A potential increase in putamen volume is an interesting phenomenon, and it may represent another factor in the development and subsequent remission of RE. To reinforce the idea of abnormal putamen development in RE, studies of normal putamen development are comparable to those seen in this studies control group.

	Anatomical region	**RE** n = 13 (mean ± SE) (mm^3)	**Control n** = 24 (mean ± SE) (mm^3)
Baseline	Left putamen	6687 ± 142	6246 ± 106
	Right putamen	6499 ± 146	6121 ± 109
Follow-up	Left putamen	6690 ± 132	6203 ± 98
	Right putamen	6521 ± 131	6040 ± 98
Rate (mm^3/year)	Left putamen	1.5	-21.5
	Right putamen	11	-40.5
SPC (%)	Left putamen	0.02	-0.34
	Right putamen	0.17	-0.66

Table 68: Measurements of putamen volume and calculation of changes in volume over two years. Similar findings to this study were seen. There was an increase in bilateral putamen volume in individuals with RE, whereas, in controls, there was a decrease. There were no significant differences between the groups for age at baseline or sex. Data extracted from Garcia-Ramos et al. (2015).

In a longitudinal study of putamen development between the ages of 7 to 24, using Freesurfer, Wierenga et al. found that putamen volume decreased in volume over time and that the peak volume of the structure would appear to be before the age of 7 (Wierenga *et al.*, 2014). Furthermore, the best model for putamen development was linear, suggesting a negative correlation between putamen volume and age. This data would indicate that the changes recorded in this study within individuals with RE are aberrant and could be involved in the process of seizure remission. Given that there is evidence of change in volume in putamen what this reflects in respect to brain development is a point of debate.

Increasing volume in the putamen could be due to an increase in the number of cortical neurons, in particular, medial spiny neurons which constitute 75-80% of all striatal neurons (Cicchetti *et al.*, 2000). There is some evidence to suggest that there is neurogenesis within the striatum from cells generated within the lateral ventricle wall of the adult human brain, but it is unclear if this occurs in adolescence (Ernst *et al.*, 2014). Another possibility for the apparent increase in putamen volume is due to a reduction in the integrity of the surrounding white matter, for example, the decrease in putamen volume in typically developing children could be due to increased white matter myelination (Sowell *et al.*, 2004).

On balance, the reduction in white matter volume or integrity seems the most likely scenario. The putamen is flanked by two large white matter tracts, on the medial side the internal capsule and the

lateral side is the external capsule. The internal capsule contains the axons of efferent motor neurons descending to the spinal cord whereas the external capsule is primarily a cortical association bundle interconnecting the frontal, insular and temporal cortices (Catani and Thiebaut De Schotten, 2008; Makris and Pandya, 2009). In the RE literature, decreased fractional anisotropy (FA) and increased mean diffusivity (MD) have been reported within the internal capsule (Kim *et al.*, 2014; Xiao *et al.*, 2014). Decreased FA and increased MD, which are both indicators of abnormal white matter structure. Furthermore, Lin *et al*., found evidence of hypertrophy in regions of the left caudate, and bilateral putamen (Lin *et al.*, 2012). Hypertrophy was greater in the anterior component of the lateral aspect of the bilateral putamen, and the medial aspect regions of hypertrophy were generally superior. Furthermore, a region of hypertrophy was seen in the inferior rostral left caudate in both the medial and lateral aspects (Figure 56). These regions border the internal and external capsules and are possible evidence of altered volume or integrity of the white matter in these structures.

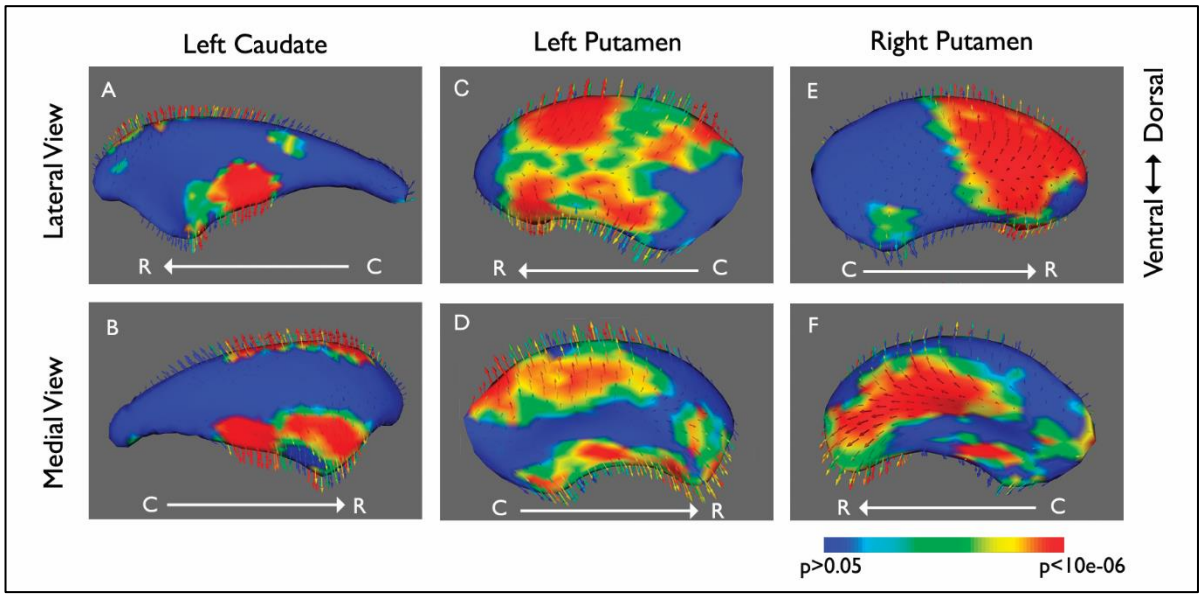


Figure 56: Regions of hypertrophy in bilateral putamen and left caudate in individuals with RE in active epilepsy. Included are maps of significant increase in subcortical structures shape. Extracted from Lin et al supplementary material.

6.5.3 Summary of conclusions

The findings of this study would suggest that there is an altered development of both cortical thickness and subcortical volume in individuals with RE compared to healthy controls. This summary will present the initial *a priori* hypotheses and whether these were answered and then the conclusions of the exploratory analyses.

To restate the first hypothesis in this study; which was there would be a delay in thinning in regions involved in the auditory-motor stream which is comprised of the superior temporal gyrus and transverse temporal gyrus, angular gyrus and supramarginal gyrus and the pars triangularis and pars opercularis. As there was no significant difference between the groups, the null hypothesis was accepted. The exploratory analysis indicates altered cortical development at both baseline and follow-up.

Exploratory analysis of cortical thickness as baseline found predominantly altered cortex in the bilateral frontal lobes with a mixture of regions of thinner and thicker cortex. Interestingly many of these regions were similar in the follow-up cohort. In addition, cross-sectional analysis in the follow-up cohort found a large region of apparently thickened cortex around the right central sulcus and postcentral gyrus. The longitudinal data also found differences between individuals with RE and healthy controls.

The area of cortical thinning was reduced and patchy in the RE group compared to the healthy controls. Despite the reduced area of thinning, a statistical comparison found large reductions in cortical thickness in the areas where thinning occurred. These reductions were seen in the left hemisphere and involved parts of the lateral orbital frontal regions, superior frontal gyrus and pre-central regions. Differences were also demonstrated in the development of the cortical and subcortical volumes.

The second and third hypotheses relate to the volume of subcortical structures. The second hypothesis was; there will be an enlarged bilateral putamen, caudate and thalamus in RE compared to controls. The analysis found no significant difference and therefore, the null hypothesis was accepted.

The third hypothesis was; between the two time-points in the RE group, there will be an increase in putamen volume, this would be greater on the right compared to the left. Whereas, in the control group, bilateral putamen volume will decrease, and this will be greatest within the right putamen compared to the left. The null hypothesis was partially rejected as an increase in putamen volumes were seen, but this was greatest on the left compared to the right. In keeping with the hypothesis, the control putamen volumes decreased in size, and this was as predicted, with the greatest change in volume in the right putamen. The exploratory analysis of sub-cortical volumes found evidence of aberrant development.

There was evidence of altered development in the intracranial volume (ICV) and non-grey or white matter. Enlarged volumes were seen in both the baseline and follow-up cohorts; furthermore, the longitudinal analysis found in the RE group that ICV and white matter volumes increased in size, whereas in the control group, they were seen to decrease.

In summary, this study has found cross-sectionally, altered development (thinner and thicker regions) within the frontal lobe both at baseline and at follow-up seizure remission. In addition, in seizure remission, there is a large region of thicker cortex over the right central regions. Longitudinally, there is increased thinning in independent regions within the left orbitofrontal, superior frontal and pre-central regions, which coincides with a bilateral increase in putamen volume.

6.5.4 **A biological explanation for findings**

As mentioned in the introduction of the chapter, the differences and change in cortical thickness or subcortical volume can be due to either change in the cortical structure or changes in the surrounding white matter. Therefore, no investigation of cortical or subcortical structure can avoid discussing this limitation. Nevertheless, there may be a unifying factor which could explain both a reduction in cortical neurons and altered development in white matter integrity. This factor could be due to altered development in the brain *in-utero*.

The cerebral cortex and striatal structures are formed from the rostral end of the embryonic neural tube; this is called the telencephalon. The dorsal telencephalon produces the cerebral cortex, and the ventral component is the substrate which generates the striatum, these regions are known as the pallium and sub-pallium, respectively (Evans *et al.*, 2012). The developing telencephalon also contains two areas called proliferative zones, where projection neurons and interneurons are born. The first area is the ventricular zone (VZ) which is positioned along the wall of the lateral ventricle and the subventricular zone (SVZ) which extends from the basal region of the VZ (Evans *et al.*, 2012). The function of these zones is behind the fate of the thickness of the cortex.

Cortical thickness is hypothesised to be regulated by the population of cells generated within the VZ and SVZ and their subsequent migration to the pial surface. It is believed that excitatory neurons are the first to arise from neuroepithelial cells of the dorsal telencephalon in the VZ. These cells then produce radial glial cells (RGC). These cells have an apical process which attaches to the ventricle and another radially orientated process which stretches out to the pial surface (Rakic, 1995). In addition, the RGCs are the principal progenitor cells for the cerebral cortex. They perform cell division which either produces two basal progenitors (BP) or a basal progenitor or a neuron/glial cell, this is known as symmetrical and asymmetrical cell division respectively (Hanashima and Toma, 2015). The BPs detach themselves and migrate into the SVZ. They can take one of two forms- either basal radial glia (bRG) or basal intermediate progenitors (bIP). These also divide into either producing two neurons or two new BPs. Generated neurons will then migrate along the RGC scaffold to produce a cortical column (Rakic, 1995; Broccoli, 1999). It may be possible that in individuals with RE that there is altered development of the cortex due to over-expression of the gene PAX6.

The over-expression of PAX6 has been associated with Rolandic epilepsy (RE) and Rolandic spikes (Panjwani *et al.*, 2016). There is evidence that a single nucleotide polymorphism (SNP) of the T allele of rs662702 disrupts the regulation of PAX6, The SNP alters the binding of microRNA-328 and is known to result in increased PAX6 expression in vitro. The PAX6 gene encodes for proteins called transcription factors which possess domains that bind to DNA to promote or enhance other genes. As a result PAX6 regulates axon guidance (Mastick *et al.*, 1997), differentiation of neurons from glia (Götz, Stoykova and Gruss, 1998),neuronal migration (Engelkamp *et al.*, 1999),the balance between pallium/subpallium volume (Carney *et al.*, 2009); arealisation (Muzio and Mallamaci, 2003); and cortical lamination (Georgala, Manuel and Price, 2011).

PAX6 is expressed at six weeks gestational age within the VZ and SVZ. In a study where PAX6 was over-expressed, there was a reduced proliferation of late cortical progenitors due to suspected negative autoregulation. Over-expressed PAX6 had the greatest effect on the rostral and central cortical regions (Manuel *et al.*, 2007). The reduction in proliferation of cortical progenitors is because PAX6 over-expression lengthens the cell cycle and promotes cell cycle exit in the late stages of corticogenesis (Georgala, Manuel and Price, 2011). The reduction in the proliferation of cortical progenitors leaves the cortical layers intact, but with a reduced number of neurons, this has the greatest effect in the superficial layers of the cortex (Georgala, Manuel and Price, 2011). Over-expressed PAX6 can also alter the differentiation of the progenitors into subtypes.

PAX6 over-expression can alter the fate of glial progenitors due to the regulation of other transcription factors. Neurog2 is a transcription factor which promotes neurogenesis, in the presence of over-expressed PAX6, this causes promotion of neurogenesis over cell renewal, favouring the overproduction of, deep layer cortical neurons such as pyramidal cells. (Sansom *et al.*, 2009). Olig2 is another transcription factor which is critical for glial cell fate determination, which is downregulated by PAX6. In the presence of over-expressed PAX6, cells expressing Olig 2, which were destined to become oligodendrocytes have an altered fate and become neurons (Jang and Goldman, 2011). The behaviour of over-expressed PAX6 is complex, but it appears to have multiple effects, some of which can be seen in the brain structure of individuals with RE.

Over-expression of PAX6 may be the factor behind altered brain structure in both the cortex and subcortical structures of individuals with RE (Figure 57). Regions of thinner cortex in the rostral and

central regions may be due to a reduction in the total number of neurons, especially in the upper layers of the cortex. Furthermore, the regions of increased cortical thickness could be due to poorly myelination as a result of a reduced number of oligodendrocytes which are required to produce glia. Longitudinally, the large changes in the reduction of cortical thickness within the rostral and central regions could occur due to myelination of these regions in effect, revealing the true thickness of the cortical matter in seizure remission. In addition, the increase in the size of the putamen could be due to loss of white matter from the axons of the large population of under-used pyramidal cells.

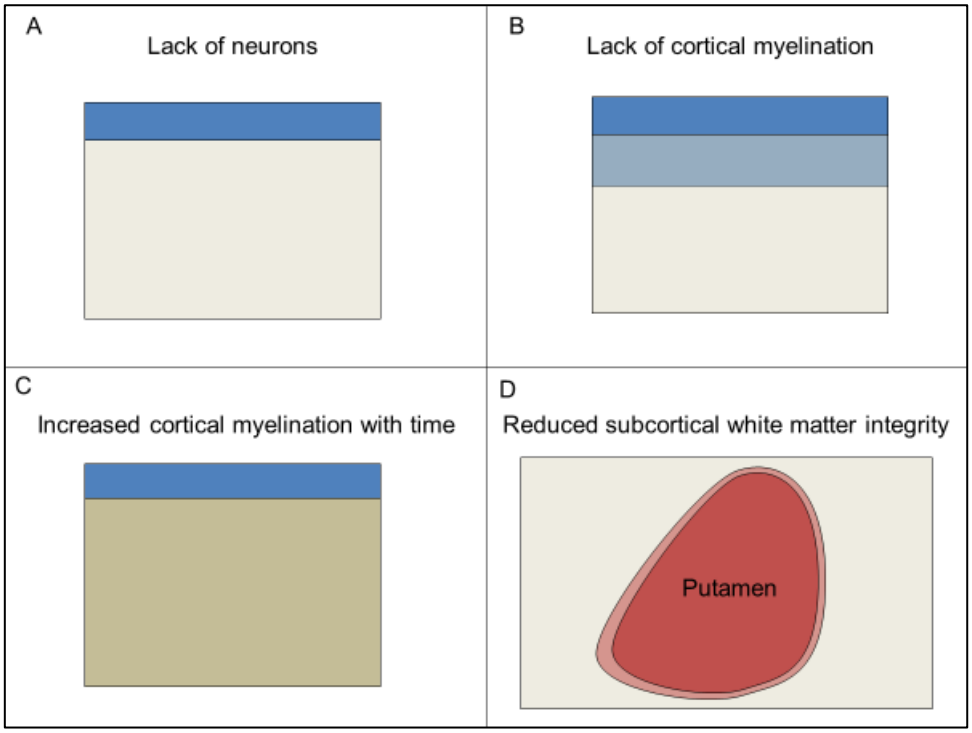


Figure 57: Theoretical explanation of altered brain structure due to over-expression of PAX6. A: A overall lack of neurons would reduce cortical thickness (blue). B: A lack of cortical myelination (cream) would increase the apparent thickness of the cortex. C: Increased cortical myelination (tan) would reveal the true cortical thickness. D: An increase in the volume of subcortical structures such as the putamen (red) could be due to a reduction in white matter integrity

6.5.5 **Participant limitations**

There are several limitations to this neuroimaging study which may have altered the findings and their interpretation. This first section discusses participant limitations.

6.5.5.1 *Hospital recruitment*

All the participants with RE were recruited from the hospital in particular tertiary healthcare facilities. Hospital recruitment can introduce a source of bias into the data collected as there is a possibility of under-recruitment from the population of individuals with RE who either did not present for medical intervention or have not required sustained treatment. In a longitudinal study, this source of bias can influence the follow-up recruitment.

6.5.5.2 *Attrition, statistical power and bias*

This study was longitudinal, and as a result, it is common for participants to drop out, in this study around 53% of individuals with RE and 55% of the controls returned, and this can lead to bias and a loss of power (Lu *et al.*, 2013). It is possible that those children with either more severe epilepsy or co-occurring cognitive problems or those from higher-income families were more likely to return. Furthermore, the reduction in numbers can reduce the studies and introduce a form of selection bias called attrition bias (Nunan, Aronson and Bankhead, 2018). Attrition bias is concerns with those participants who do not return and the reasons why. A loss of 5-20 % can lead to attrition bias furthermore as this can lead to an effect of non-random sampling. This effect can alter the mean and variance of the data (Schulz and Grimes, 2002). Regarding attrition bias, there were multiple reasons for participants not returning, and this included not tolerant of scanning, fixed orthodontic prosthesis, not wanting to be involved as they were in seizure remission and did not want to take part in epilepsy research and those that were uncontactable. Nevertheless, there was no review on which participants

returned, and this would be needed for a true understanding of the data. To counter this problem, to see if any bias could be revealed, cross-sectional measures were reported.

6.5.5.3 *Sex differences*

Despite the evidence that individuals with RE are more likely to be male, the study was not stratified, and this may have produced some bias (Panayiotopoulos *et al.*, 2008). However, the proportion of the sexes was statistically assessed, and sex was used as a covariate in many of the analyses. Furthermore, a longitudinal study by Wierenga found in 49 typically developing children that changes in cortical thickness were not affected by the sex of the individual. Whereas, there was a sex effect on the cortical volume and surface area (L. M. Wierenga *et al.*, 2014). It is important to note, however, that cerebral white matter volumes and putamen size had greater variability in males (Wierenga *et al.*, 2018), which may have influenced the finding in those individuals with RE.

6.5.5.4 *The statistically significant age difference between the cohorts*

There is a significant difference in age between the two groups (Baseline p=0.026 and follow-up p=0.007), which can affect the interpretation of the results. In the literature on changes in cortical thickness, a common theme is a linear decrease in thickness with age (L. M. Wierenga *et al.*, 2014). Therefore, it would be prudent to expect in the RE group, who are younger, that the cortex would be thicker and thus significantly different. This study has demonstrated little difference in cortical thickness at a hemisphere or cortical parcellation level, which means on the whole that there is great similarity between the groups despite the significant age difference. Furthermore, even though the longitudinal data demonstrated evidence of cortical thinning in both groups, the same phenomena of non-significant difference in the hemisphere or most parcellation thickness persisted in both the baseline and follow-up cohorts.

6.5.5.5 *The side of the Rolandic spike*

Spike side and evidence of status epilepticus were reported. Despite this, the information was not utilised in analysis and thus may have influenced the results. Ideally, if the study had large enough numbers, the analysis could have been stratified by spike side. However, there is uncertainty about the effect this analysis would have because the Rolandic spike is known to migrate between studies (Ewen *et al.*, 2011). Evidence of an episode of status epilepticus may have altered the findings.

6.5.5.6 *Experience of status epilepticus*

Status-epilepticus (SE) in RE, is reported to be rare. Wirrell *et al.* in their study of 42 children with RE found around 7% of the children with RE had experienced SE (Wirrell *et al.*, 1995). The Wirrel study found a small cohort with SE compared to this study, where 20% had experienced SE. However, it is important to note that the Wirrel study was cross-sectional and collected from hospital case notes over two years whereas in this study the children were followed up and parents were asked about SE at both baseline and follow-up. Therefore, the methodologies of this study may have increased the detection of SE in this patient group and may be more prevalent than previously thought. It also appears that SE in RE has a different quality to SE in other epilepsies.

Status epilepticus in children with RE is varied in duration, easily treated and has a good prognosis (Fejerman, 2009). Fejerman states that some status episodes with include focal motor seizures of the face and anarthria can last for days or weeks. In comparison, this study reports the longest seizure duration as 2.5 hours. According, to the ILAEs definition and classification of SE a seizure of this duration could have long term consequences such as neuronal death, neuronal injury and the alteration of neuronal networks (Trinka *et al.*, 2015). Despite this, all individuals with RE with SE in this study survived their experience of status and did not have any resulting disabilities. Indeed, there is little worry about SE in RE as the previously mentioned ILAE guidelines do not acknowledge it. This does not mean that there is a disregard for those individuals with RE and SE, and it does warrant further investigation.

6.5.5.7 *Changes in participant in scanner behaviour and movement artefacts*

A valid criticism of this study is that apparent changes in cortical thickness between baseline and follow-up may be influenced by the amount of movement artefact in the scanner. This is because younger children are more likely to move in the scanner compared to older children and adolescents (Dean *et al.*, 2014). As the author was aware of this problem, all scans were visually assessed and then processed using the ENIGMA protocol as detailed in this Chapter. The author believes that these measures are more than adequate to address concerns about movement artefact.

6.5.6 **Technical limitations**

In the study, there were several technical limitations.

6.5.6.1 *Freesurfer intra-cranial volume and bias*

Freesurfer may produce bias in its calculation of intracranial volume (ICV). It is important to understand that Freesurfer does not calculate ICV by counting the number of voxels within the skull. Automated voxel counting would be the best method; however, CSF and skull are both dark on a T1 image, and this would confuse the algorithm. As a result, Freesurfer uses an alternative method of using the scaling factor which is used in the linear transform to the MNI305 space. As a result, the intra-cranial volume value is biased by total brain volume (Klasson *et al.*, 2018). Nevertheless, Freesurfer has been reported to have Pearson correlations between 0.89 and 0.94 when compared with manually estimated intracranial volume (Malone *et al.*, 2015). Furthermore, the biased ICV technique has been used in both groups, thus reducing the bias effect.

6.5.6.2 *Freesurfer Qdec Monte-Carlo simulation*

A Monte-Carlo simulation was used to correct for multiple comparisons in the vertices analysis; this method, however, can produce problems in interpretation. The problem lies in the smoothing of the data as the smoothing results in larger clusters. Smoothing is a minor problem in the uncorrected data as a range of clusters with p-values from 0.001 to 0.05 can be viewed. It becomes a bigger problem after correction at $p = < 0.05$ as the clusters become large, and this can hide highly significant areas. Therefore, it is important to keep in mind that within the corrected clusters are areas of high significance. To mitigate the problem, the vertices of greatest significance were reported, in addition to presenting both the uncorrected and corrected results.

6.5.6.3 *Different scanners at baseline and follow-up*

One large limitation of this study was the use of different scanners in the baseline and follow-up cohorts. An ideal MRI experiment would utilise the same scanner in the same location due to less change in the B0 and the ability to use the same scanning protocol. In this respect, the study failed. However, it is important to note that all participants scanned at baseline were scanned in the same scanner, and all participants scanned at follow-up were scanned in the new scanner. In effect, they are cancelling out the variation of the B0 and scanning protocols in both the cross-sectional and longitudinal analyses.

6.5.6.4 *Comparisons difficult to make due to limited 3T studies*

Comparing the findings of this study with other studies in the literature could be difficult due to the use of a scanner with a 3 Tesla (T) magnetic field strength. The sole, longitudinal study in the literature is by Garcia-Ramos, and they used a 1.5 T scanner (Garcia-Ramos *et al.*, 2015). Other longitudinal 3T studies are critical for the understanding of these results, as field-strength can alter the measurement

of subcortical volumes (Jovicich *et al.*, 2009). Despite the discrepancy with the aforementioned study, three cross-sectional studies utilised a 3T scanner and Freesurfer measures of cortical thickness (Geke M. Overvliet, Besseling, Jansen, *et al.*, 2013; Pardoe *et al.*, 2013; Kim *et al.*, 2015) which may help in understanding the findings of this study.

7 Changes in scalp electroencephalogram in children with Rolandic epilepsy between active epilepsy and seizure remission

7.1 Introduction

7.1.1 Electro-encephalogram in Rolandic epilepsy

Rolandic epilepsy is an idiopathic focal epilepsy of childhood where seizure remission is close to a certainty. A meta-analysis conducted by Bouma et al., found that in children with a diagnosis of RE, 50% are in remission at the age of 6 years, 92% at 12 years and 99.8% at the age of 18 years (Bouma *et al.*, 1997). The time to seizure remission is relatively fast, in a mixed cohort of 29 children with typical and atypical RE it took from the first seizure an average of 2.7±3.7 years to achieve seizure remission (Callenbach *et al.*, 2010). In the literature, the time to seizure remission is well defined, but there is a lack of clarity regarding the remission of inter-ictal and background abnormalities on the scalp electroencephalogram (EEG). To understand what inter-ictal EEG abnormalities represent requires an understanding of how the scalp EEG is recorded.

The EEG is a tool which can be used to record neurophysiological abnormalities in children with epilepsy. Multiple electrodes are placed equidistant across the surface of the scalp to record changes in voltage over time, known as the EEG. Current evidence would suggest that the EEG signal is generated from the summation of post-synaptic potentials which are produced by the apical dendrites of pyramidal cells within layers III, IV and VI of the cortex (Tufenkjianad, 2017). The excitation of the post-synaptic membrane in apical dendrites leads to an influx of sodium ions into the dendrite and leads to depolarisation and the generation of a dipole potential (Tufenkjianad, 2017). The generated potentials will only be recorded as an EEG signal, if they are synchronised, perpendicular to the scalp surface and occur in an area of the cortex ≥ 6 cm^2 (Olejniczak, 2006; Tufenkjianad, 2017). Furthermore, to reach the scalp, the potential must pass through several layers of tissues with different electrical properties and complex geometry. As a result, the potential is transformed and attenuated in amplitude (Lopes da Silva, 2013). EEG has its limitations, but it is a relatively good measure of cortical function, in particular, it is very good in identifying abnormal brain activities such as the Rolandic spike (RS)

The typical inter-ictal discharge in rolandic epilepsy is the rolandic spike (RS) (Kellaway, 2000). RS are said to represent an electrochemical imbalance between inhibition and excitation. Their duration is generally around 74 ms and therefore is best classified as a sharp wave rather than a spike. The sharp morphology would indicate a distribution of an extensive neuronal source which has a slight delay in synchronisation (Kellaway, 2000). The dipole of the spike varies from child to child; indeed, some children do not have defined dipoles (Gregory and Wong, 1992). Similarly, RS can be generated in a multitude of different cortical locations.

RS are predominantly seen over the centro-temporal regions lateralised, bilateral either independently or synchronously, and it is common in bilateral discharges to have a side emphasis where the RS have greater prevalence (Holmes, 1993). Spikes can also be seen outside the centro-temporal regions, in the parietal, occipital and even frontal regions (Panayiotopoulos, 2010). In some individuals with RE, their RS can migrate across the scalp, this has been reported and measured in separate studies by Kellaway and Ewen (Kellaway, 2000; Ewen *et al.*, 2011). Migration of focal spikes in focal epilepsies appears to be unique to children and is a possible sign of good outcome as fixed spikes can indicate fixed lesions (Lee, Chen and Lee, 2010). Indeed, the migratory behaviour of RS would suggest that they are being generated by a dynamic hyper-excitable cortex which may be reflective of a developmental process. Another development feature of RS is their remission with age.

The appearance and disappearance of RS in individuals with RE appear to be related to age. In a large study by *Kim et al.*, they found that the mean age of spike remission was 11.89±2.11 years, age of spike remission positively correlated with age of seizure onset with spikes persistence greatest in individuals with an earlier onset of epilepsy (Kim *et al.*, 2018). The relationship between spikes and seizure onset would suggest that the spikes are age-related in appearance rather than associated with the onset of seizures. In addition, the same study found that withdrawal of medication after two years of seizure freedom regardless of the presence RS did not lead to any seizure reoccurrence. These findings propose uncertainty around the relationship between RS and seizures this is a common feature in literature, as the RS is not unique to RE and could be said to be an EEG feature of neurodevelopmental disorder, regardless of seizures.

The rolandic spike had been implicated as a marker of impaired brain maturation. It can be seen in many different neuro-developmental disorders such as attention deficit hyperactivity disorder (ADHD)

(Silvestri *et al.*, 2007), developmental coordination disorder (DCD) (Scabar *et al.*, 2006), developmental language disorder (DLD) (Selassie, 2010) and autism (Ghacibeh and Fields, 2015). Indeed, in children with RE, there are features of these disorders in their co-occurring cognitive problems (See Chapter 1). Recent research has pointed towards a dysregulation in the expression of the PAX6 gene, and it is it thought that this is altering the brain development in individuals with RE and contributing to the generation of seizures (Panjwani *et al.*, 2016). As RS may not be the best marker for seizures, it may be that quantitative analysis of the resting state EEG might provide extra information about the epileptogenic process.

Resting-state EEG is recorded at rest in the absence of a task; it can be recorded when the eyes are open or when they are closed (van Diessen *et al.*, 2015). There has been recent interest in the power and frequencies of resting-state EEG in epilepsy. Peak low alpha frequencies (6-9 Hz) could be a potential biomarker of the disorder. In an individual with epilepsy, this frequency band has been reduced compared to controls (Schmidt *et al.*, 2016; Abela *et al.*, 2018). This feature has been found in RE, Adebimpe *et al.* found that dominant peak frequency was reduced compared to age-matched controls (Adebimpe *et al.*, 2015). The reduction of dominant peak frequency would suggest that individuals with RE may have some feature of delayed development. Topographic analysis of resting-state EEG power has also found differences.

There is evidence of increased absolute and altered relative power in individuals with RE compared to controls. Braga *et al.* found evidence of increased absolute power in all recorded EEG channels compared to healthy controls (Braga, Manzano and Nóbrega, 2000). Increased relative theta power has been found in the right centro-temporal and bilateral frontal and parieto-occipital areas compared to healthy controls. Whereas, relative alpha (8.5-13 Hz) and beta (13.5-30 Hz) power tended to decrease over the same regions (Adebimpe *et al.*, 2015). There is evidence of increased absolute power and altered relative power, which would suggest altered brain function due to development. The effect of age and duration of seizure remission on EEG power in individuals with RE further supports a developmental relationship.

There are a few studies which detail changes in EEG power with age in individuals with RE. Braga *et al.* found, that there when comparing a 7-9 years group with RE with age-matched controls. Absolute power was larger than controls across all frequency bands in a generalised distribution. In another

group aged 10-12, absolute power was only increased across the majority of the electrodes in the theta and delta frequencies. In keeping with a decrease in power with age, Song *et al.* found evidence of a negative correlation between global absolute power and age. Specifically, absolute alpha power demonstrated a significant negative relationship with the duration of seizure freedom. Moreover, the same relationship was seen in absolute beta power but within the lower part of the sensorimotor cortex (Song *et al.*, 2019). The evidence on EEG would suggest that it decrease with age and into seizure remission.

In summary, in children with RE compared to controls in addition to RS, there is evidence of reduced peak alpha frequency, increased global power and altered relative power in the frontal, central and parietal regions. Global absolute power decreases with age or with the duration in seizure remission. There is no body of evidence on changes in relative power in individuals with RE.

7.1.2 Electro-encephalogram in healthy children

During the development of child to adolescent, there is substantive evidence which indicates changes in the resting state EEG. In children, total and frequency band absolute EEG power declines with an increase in age (Segalowitz, Santesso and Jetha, 2010). Other studies found that the change in EEG was more nuanced and regional. High power frequencies such as delta and theta would decrease in power with increasing age (Uhlhaas *et al.*, 2010; Miskovic *et al.*, 2015) (Figure 58). Posterior alpha band power and frequency would also increase with some studies indicating the maturation of alpha frequencies within adolescence (Marcuse *et al.*, 2008). Topographical changes in EEG power have been documented in healthy children.

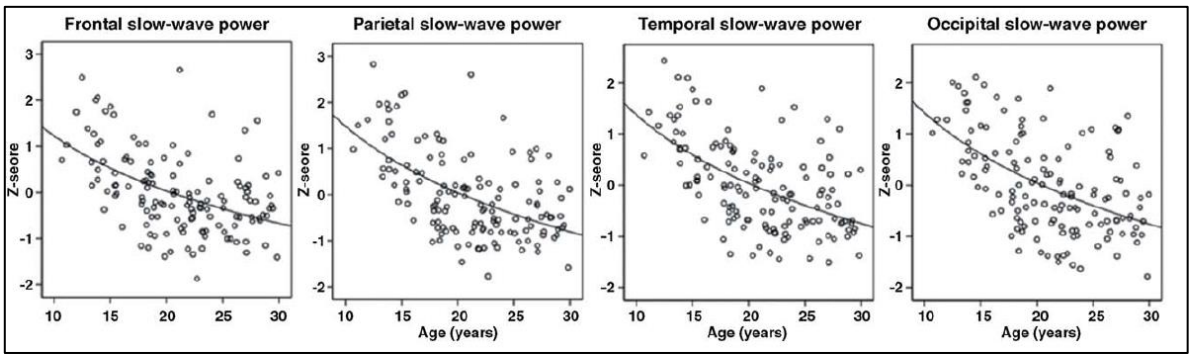


Figure 58: Correlations of absolute slow frequency power with age in healthy controls. Slow frequencies included 0.5-7.5 Hz recorded in individuals between 10-30 years of age. Data recorded from temporal and occipital electrodes. These graphs deomstrate a global decrease in delta power with age. Extracted from Ulhaus et al (2010)

Changes in EEG power with age occurs with a posterior to anterior axis. A study found that there was a substitution of slow-wave (delta and theta frequencies) power with faster activities which proceeds from posterior to anterior sites (Segalowitz, Santesso and Jetha, 2010). Another study replicated this finding, in addition, relative alpha and beta power increased with age across the scalp, with greatest relative alpha power change posteriorly and relative beta power in the right frontocentral regions (Cragg *et al.*, 2011). These changes appear to be related to developmental changes in the brains structure and function.

Changes in the resting state EEG between childhood and adolescence are considered to reflect developmental changes in the structure and function of the brains grey and white matter. In the grey matter, this may be due to reductions in cortical thickness due to cortical pruning (Whitford *et al.*, 2007) and reduction in synaptic density (Segalowitz, Santesso and Jetha, 2010). The altered cortical function may be due to altered neurotransmitter levels such as gamma-aminobutyric acid (GABA) (Silveri *et al.*, 2013) and dopamine (Wahlstrom *et al.*, 2010). Changes in white matter may be behind the increases in alpha frequency and amplitude. It is postulated that this is due to improved myelination and an increase in axon diameter, which results in the increased speed of action potentials (Segalowitz, Santesso and Jetha, 2010).

This introduction has demonstrated that EEG can provide a range of different measures to measure brain function in children with RE. This chapter will analyse EEG data from individuals with RE with qualitative and quantitative methods to further the understanding of brain development and function between active epilepsy and seizure remission. The quantitative analysis will help in the development of *a priori* hypotheses for future controlled studies.

7.1.3 **Hypotheses**

The following qualitative hypotheses will be explored;

i. Remission of rolandic spikes will occur after the remission of seizures. Bouma *et al.* identified that 10% of children with RE in seizure remission had RS, but they did not compare this with age or duration of seizure remission. Therefore, it is pertinent to know whether a group of participants with no evidence of RS in seizure remission have a greater period of seizure remission?

ii. There is evidence for and against the suppression of RS with pharmacological therapy. In particular clobazam, clonazepam and levetiracetam are likely to suppress spikes in RE (Mitsudome *et al.*, 1997; Bakke *et al.*, 2011) but there is evidence that most drugs do not (Kim *et al.*, 2018). Do those individuals with RE in seizure remission and using pharmacological therapy have an absence of RS?

Changes in EEG power are proposed to be related to developmental changes in the structure and function of the brain. To investigate whether there is altered development in RE the following quantitative hypotheses will be explored:

i. What is the change in global peak dominant frequency between active epilepsy and seizure remission?

ii. Does global EEG power decrease as the child enters seizure remission and if so, by how much?

iii. What is the change in relative power in each frequency band between active epilepsy and seizure remission and how does this relate to time from the last seizure?

iv. Topographic changes in EEG power have been demonstrated in healthy controls. Are there similar changes in the topography of EEG spectra between active epilepsy and seizure remission?

7.2 Methods

Participants were recruited for a longitudinal study in 2012. To qualify for the baseline, they required a diagnosis of RE, which fulfilled the ILAE criteria for the classification of epilepsies (Engel, 2001) and were having active seizures. Central-temporal or rolandic spikes needed to be apparent on one of their previous EEGs. Furthermore, their recorded seizures required a focal component which included both sensory and motor phenomena. Participants were included if they were experiencing co-occurring cognitive problems such as dyslexia, ADHD, DLD and autism spectrum disorder. Despite this, they were required to have a WASI IQ score greater than 80 at baseline. Participants were not included if they only experienced one seizure; they had an abnormal MRI, an abnormal EEG background, such as continuous focal slow, which is not in keeping with typical RE.

To qualify for the follow-up, the participants had to fulfil one of the criteria: seizure remission for longer than six months or older than 15 years. Seizure remission could be with or without pharmacological intervention. A single “breakthrough seizure” within the last six months before the recording was still counted as seizure remission if the individual had the previous year seizure-free. The returning participants were excluded if their diagnosis had been changed since the baseline or there had been an evolution of the epilepsy.

7.2.1 **Qualitative**

7.2.1.1 *Baseline EEG reports*

EEG reports for each participant were retrospectively collected from the recruiting hospitals in England. The recruiting hospitals included Kings College Hospital, Evelina Children Hospital, Brighton and Sussex University Hospital, Chelsea and Westminster Hospital, The Whittington Hospital, Luton and Dunstable Hospital, Oxford Radcliffe Hospital and Taunton and Somerset NHS Trusts. The EEGs were recorded using either XLTEK, Nicolet or Nihon Koden EEG machines. The reports were a mixture of clinical routine and sleep-deprived EEG recordings and included photic stimulation and hyperventilation activation procedures. Some of the sleep recordings were drug-induced with melatonin. All reports contained a technical report by a clinical physiologist and consultant neurophysiologist's clinical opinion. There was difficulty in obtaining every EEG report in chronological order for each participant. Nevertheless, the age of the child when the EEG was recorded was more important than obtaining every EEG. As a result, the first EEG recordings in this study may not be the first EEG.

All reports were included in the analysis if the participants had responded to the follow-up and seizure remission was reported. Furthermore, copies of the raw EEG data were included. The date of the test and spike locations were identified from the reports and collated. The spike location was simplified into one of five categories: Absent, Left, Right, Bilateral-Left emphasis, Bilateral-Right emphasis and Bilateral.

7.2.1.2 *Follow-up EEG recording*

Participants in seizure remission who returned for the follow-up were invited to have a sleep-deprived EEG. The sleep deprivation was mild; the adolescents were told to go to bed later or wake up earlier than usual to increase drowsiness during the recording. This technique was utilised to increase the likelihood of the participants entering a sleep state and thus obtaining the optimal state for recording RS (Blom and Heijbel, 1975). Excessive sleep deprivation was avoided due to the risk of generating seizures (Schmitt, 2015). To capitalise on sleep deprivation and to control for circadian influence all EEG recordings were performed in the morning before midday.

The follow-up EEG was recording using BrainAmp MR EEG system with a BRAINCAP MR electrode cap hardware and Brain Vision recorder software (Brainproducts, Germany). The electrode cap contained 74 equidistant electrodes, which are distributed across the scalp using the international 10-10 system (see Figure 59). At each electrode site, the scalp was prepared with alcohol to clean the skin, and a conducting gel with abrasive properties (ABRALYT 2000) was applied with a cotton bud. Finally, the conducting gel was injected into each electrode position. All electrode impedances were

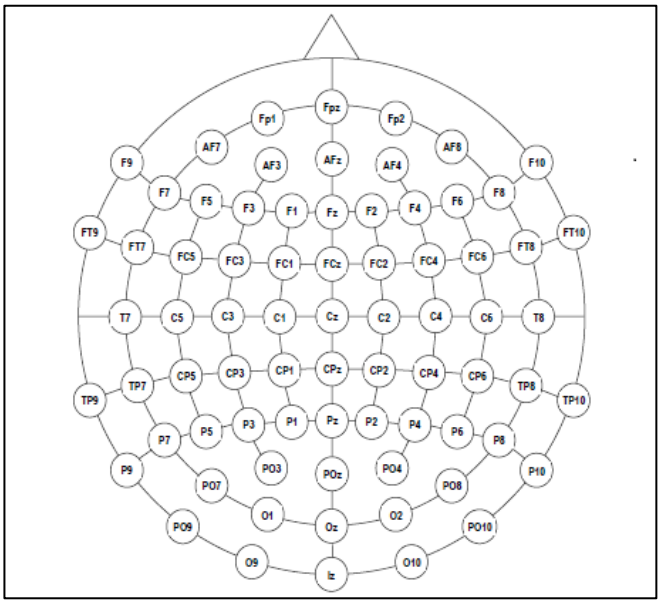


Figure 59: Schematic of the 10-10 electrode system used for the recording of high-density EEG. The anterior is the top of the diagram. This diagram displays the equidistant electrode locations for seventy-four electrodes. The ground electrode was located at AFz and the reference at FCz.

below 10 KΩ. The EEG sampling frequency was 30 kHz, with a low-frequency filter of 0.5 Hz and a high-frequency filter of 70 Hz.

The recordings were performed within a room electrically isolated within a Faraday cage, furthermore, to minimise alternating current electrical interference the EEG equipment was battery powered. All recordings were performed in a supine position on a soft bed. The recording was an hour in duration, regardless of whether the participants entered a sleep state. At the beginning of the recording, participants were told to “lie with their eyes closed and if they wanted to, they could have a nap”. Clinical activation procedures, such as intermittent photic stimulation or hyperventilation, were not used.

The EEGs were visually assessed to see if spikes were apparent and their location was recorded. The visual assessment was performed by the author and abnormalities were confirmed by a consultant clinical neurophysiologist, Prof Michalis Koutroumanidis. The same coding of spikes used in the analysis of the EEG reports was used. Any other abnormalities such as generalised EEG phenomena were reported. The data was combined with the retrospective EEG report data to produce a qualitative timeline of EEG abnormalities against age and duration of epilepsy.

7.2.2 **Quantitative**

Baseline clinical EEG data were collected from the recruiting hospitals. All of the EEGs were acquired using referential format; it was not documented in the reports where the reference was located, however international guidelines state that the reference “should be placed in a location that is not susceptible to an artefact. An extra midline electrode is suitable” (OSET, 1999). Follow-up, non-clinical EEG data was collected in seizure remission as detailed (section 7.2.1.2).

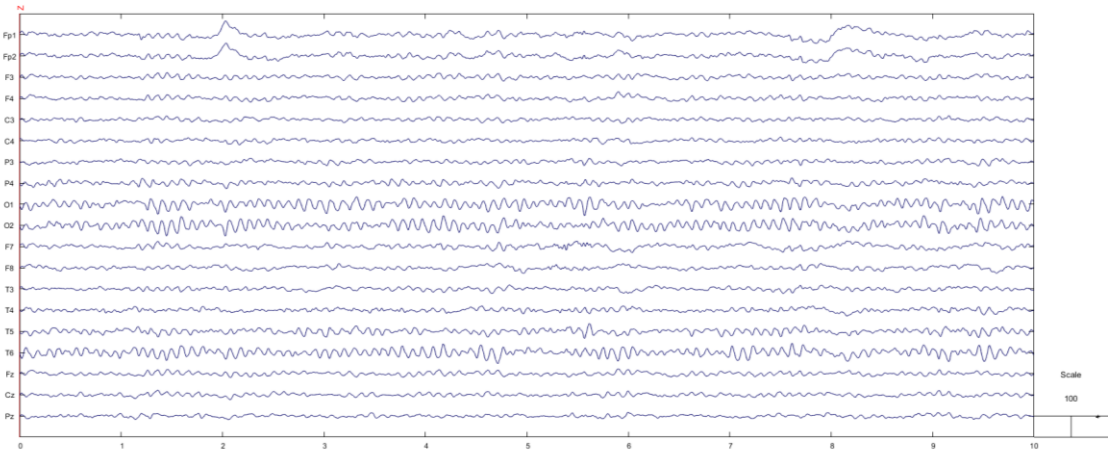


Figure 60: Example of a 10-second resting state epoch. Included are 19 electrode channels on a 10 second time-base with a sensitivity of 100 µV per cm. Alpha frequencies (>8Hz and <13 Hz) are predominantly seen on the O1, O2 and T6 channels. Any epochs with eye movements or excessive electro-myographic artefact were not included.

Anonymised EEG data was received from the recruiting hospitals on encrypted universal serial bus (USB) stick. EEG files were collected in either .edf (European data format) or were converted to a .edf file type. Using EEGLAB and a word document with the time-marker locations, a single ten-second epoch of resting-state EEG was extracted from the EEG data. In this study, resting state, EEG is background EEG when the participant’s eyes are closed, eye movements are absent, and abnormalities are absent (Figure 60). These epochs were sampled from the follow-up data preceding the sleep state if one occurred. The epochs were pre-processed using EEGLAB (Delorme and Makeig, 2004) and Matlab 2018b software package. To provide standardisation between the baseline and follow-up EEG data, the number of electrodes was reduced to 19. The same electrodes were

analysed at both baseline and follow-up these included; Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1 and O2. The EEG data was then standardised between the time-points.

To standardise the EEG data, the mean EEG signal was extracted from each channel, and then the data were re-referenced to an average reference. The average reference is a sum of the potentials across all of the electrodes divided by the number of electrodes. The data were downsampled to 250 Hz, and linear bandpass frequency filters were applied. The low-frequency filter had a cut-off of 0.5 Hz, and the high-frequency filter had a cut-off at 40 Hz. The low-frequency filter was used to remove slow-wave sweat artefact, and fast frequency filter was used to remove electro-myographic (EMG) artefact.

All 19 electrodes were averaged in each participant, and the Fast Fourier transform (FFT) function was used to produce a spectrogram of average global absolute power. The FFT used a Hamming window of 1 second with a 50% overlap. The FFT results were presented as a graph of power over frequency. The dominant global frequency was defined as the highest peak of absolute power within the theta and alpha frequency bands. This peak was visually identified and measured using Matlab datatips graphical function (Figure 61).

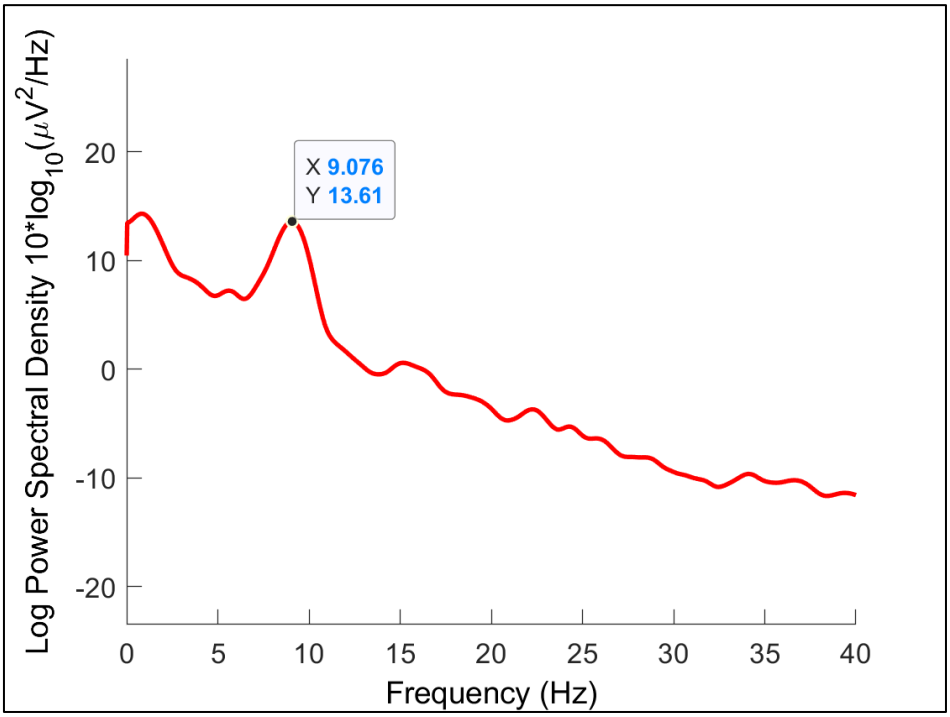


Figure 61: Spectral frequency of resting state EEG in a participant with RE. The graph is shows global changes in log power spectral density with increasing frequency. The global dominant frequency (GDF) is measured using the MATLAB data-tips function.

Furthermore, the average absolute power spectral density for frequencies ranging between 1-40 Hz and for five separate frequency bands was calculated from the EEGLAB spectopo output of the 19 averaged electrodes. The frequency bands included delta (1-3 Hz), theta (4-7 Hz), alpha (8-13 Hz), beta (14-30 Hz) and gamma (31-40 Hz).

The spectopo output produced a measure of power in $10*\log_{10}(\mu V^2/Hz)$. To obtain the absolute power, the spectopo output was divided by ten and converted with inverse log to produce measures of power in $\mu V^2/Hz$. The results for each frequency band were integrated and then combined to produce the total absolute power of the signal. Relative power was calculated by dividing the absolute power of each frequency band by the total power and multiplying the results by 100 to achieve a percentage. The relative power calculation reflects the relationship between frequency bands.

Finally, topographic maps of absolute power between 1-40Hz were created using the EEGLAB function topoplot. The electrode locations were standardised between participants using the EEGLAB

inbuilt BESA 4-shell dipfit spherical model. Using these positions, a topographic scalp map of EEG absolute power was constructed using co-interpolation of the data on a fine Cartesian grid. A separate image was produced from 1-40 Hz in 1 Hz increments. Each image contained three maps; the baseline data scalp map, the follow-up data scalp map and a statistical comparison corrected for false discovery rate on a scalp map. The baseline and follow-up data were heat maps constrained to the same power scale, whereas the statistical maps were colour scaled by p-values.

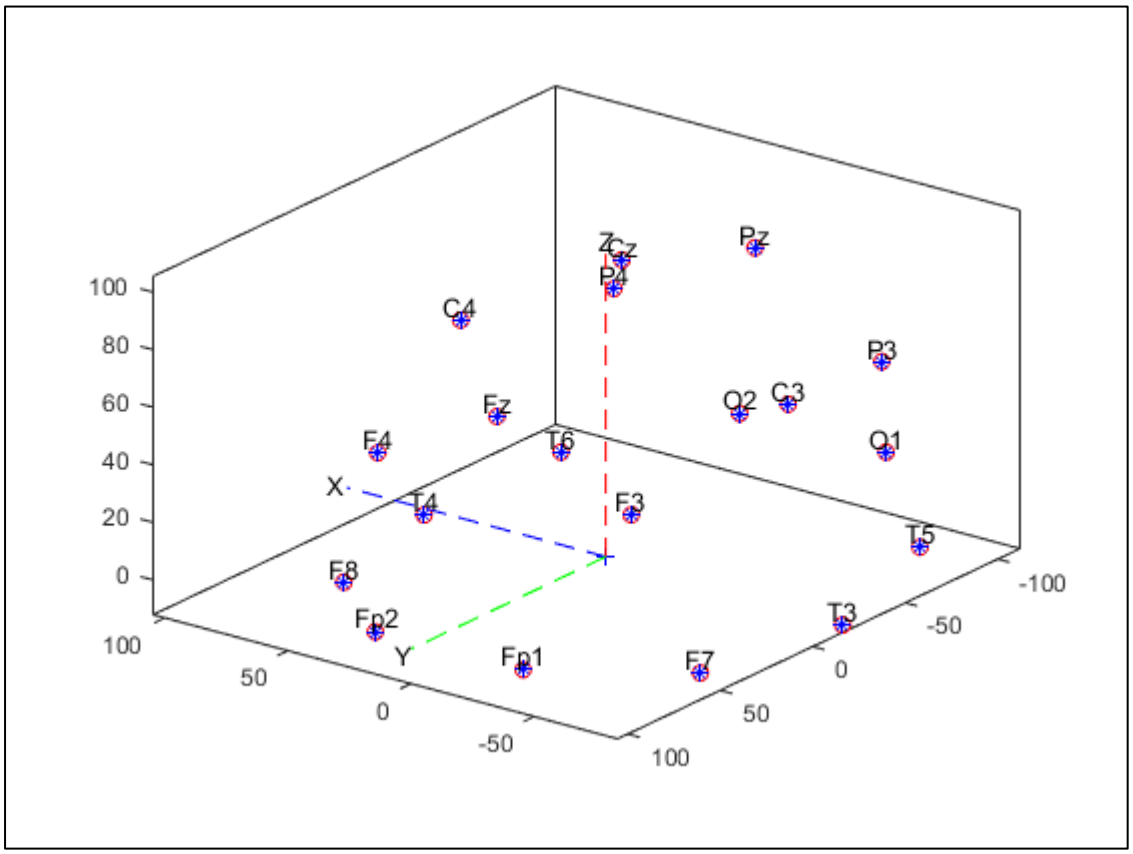


Figure 62: Electrode locations within the three-dimensional BESA spherical model. Nineteen electrodes are presented within a fine Cartesian grid which is comprised of a Y, X and Z axes. All of these electrodes were used in the generation of the grand average power spectra and topographic maps.

7.2.3 **Statistical analysis**

7.2.3.1 *Qualitative*

Using SPSS Version 25 (IBM Corp), the qualitative data were categorised and compared with demographic data using t-tests or MANOVA models. The model was corrected for age and sex.

7.2.3.2 *Quantitative*

Unpaired t-tests were used to analyse dominant global rhythm, absolute and relative power between active seizures and seizure remission. Q-Q plots were used to assess the departure of residuals from normality. If the Q-Q plots were not normal in distribution, the data was log-transformed. Equality of variance was assessed using Levene's test. Bonferroni correction was used in the analysis of the absolute and relative power of frequency bands between the two time points. Linear regression was used to understand how an increase in age, related to changes in the dominant global frequency (GDF), total absolute EEG power and the relative EEG power within each frequency band. Furthermore, exploratory analysis of relative EEG power and time to final seizure used a complex hierarchical linear regression with a backward stepwise method. Comparisons between global power spectra and topographic power maps between baseline and follow-up up were made using the unpaired t-test function in EEGLAB. All results were corrected for family-wise error (FWE) with a false discovery rate (FDR) correction ($p-<0.05$).

7.3 Results

7.3.1 Qualitative Analysis

7.3.1.1 *Rolandic spikes in active epilepsy and follow-up*

In total, thirty-four EEG reports from 22 participants were retrospectively collected. Sixteen individuals responded to the follow-up to provide a final seizure date, which resulted in 28 reports being used for the analysis. Fourteen individuals had a follow-up EEG in seizure remission. The demographics for these individuals are displayed in Table 69.

	N	Age (years)	Sex (%)	Handedness (%)	Onset (years)	AED (%)	Sleep (%)
1st	16	8.10±2.5	62.5	93.75	7.11±2.16	43.8	50
2nd	7	7.44±2.55	71.4	100	6.85±1.38	14.3	85.7
3rd	4	11.0±0.89	75	100	7.0±1.75	25	80
FU	14	14.19±2.14	64.28	93.75	6.95±2.19	42.9	85.71

Table 69: Demographics of participants in each group at first, second, third and fourth EEG recording.

All participants in this study were in the 1st EEG group, and a proportion of the 1st EEG group was in the other groups. All participants in the follow-up group (FU) were in the 1st EEG group. Included are Age: mean age, Sex: percentage male, handedness: percentage right-handed, Epilepsy onset: age of first Rolandic seizure, AED: on medication during the recording and Sleep: Whether the individual attained a sleep state during the EEG.

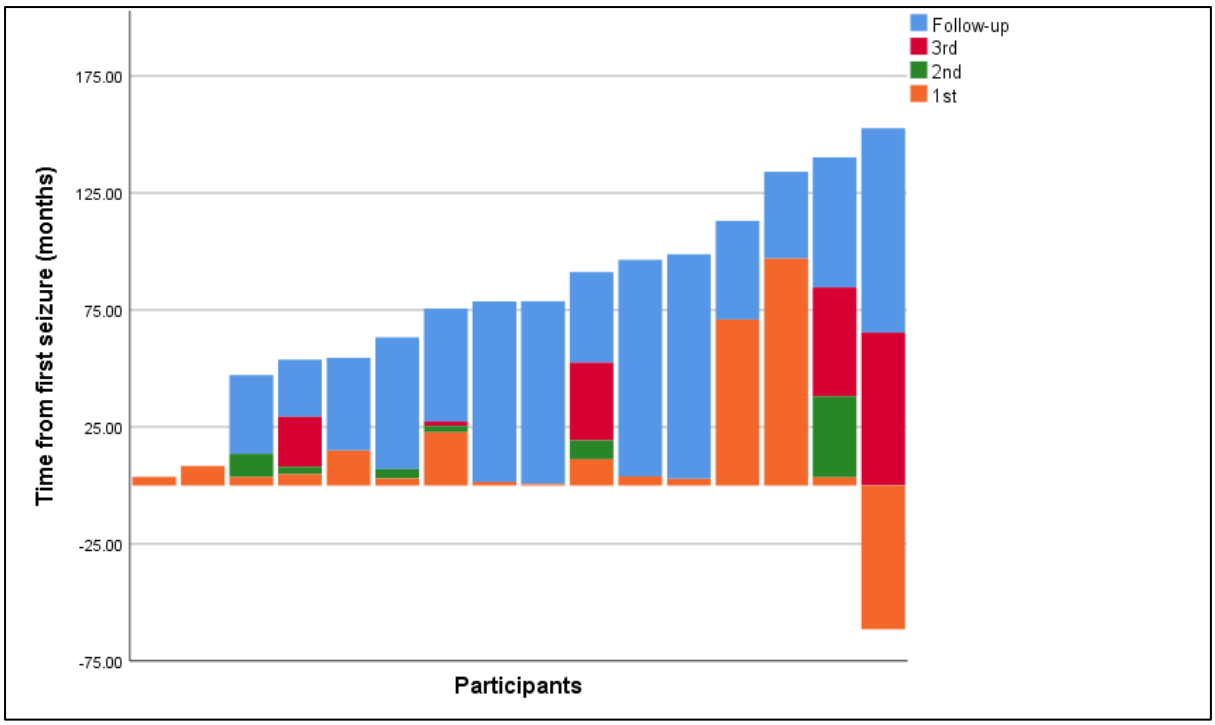


Figure 63: Time in months from first seizure to EEG recording. The top of each bar represents the time of the EEG recording. Each colour represents a different EEG. Orange: First EEG, Green: Second EEG, Red: Third EEG and blue: follow-up EEG in seizure remission. Zero on the Y-axis is the time-point of the first seizure. Note that one individual had an EEG before their first seizure.

The majority of the participants had more than one EEG recording. Two had one recording in active epilepsy and no follow-up. Including the follow-ups EEG, seven participants had two recordings, two had three EEG recordings, and four had four EEG recordings. All of the participants, except for one, had their first EEG after their first seizure. The exception was a participant who had an EEG for sleep problems, with no documented seizures at the age of 2.28 years; their EEG at the time contained right-sided spikes. At the age of 7.4 years, he experienced his first Rolandic seizure. The statistics for those participants who had their first EEG after their first seizure. The mean time to EEG was within 16.15±25.7 months of their first seizure with a mean age of 8.8±2.07; three participants had EEGs ≥50 months after their seizure onset. These children had EEGs, closer to the onset of seizures, but there was difficulty in acquiring the reports from the recruiting hospitals.

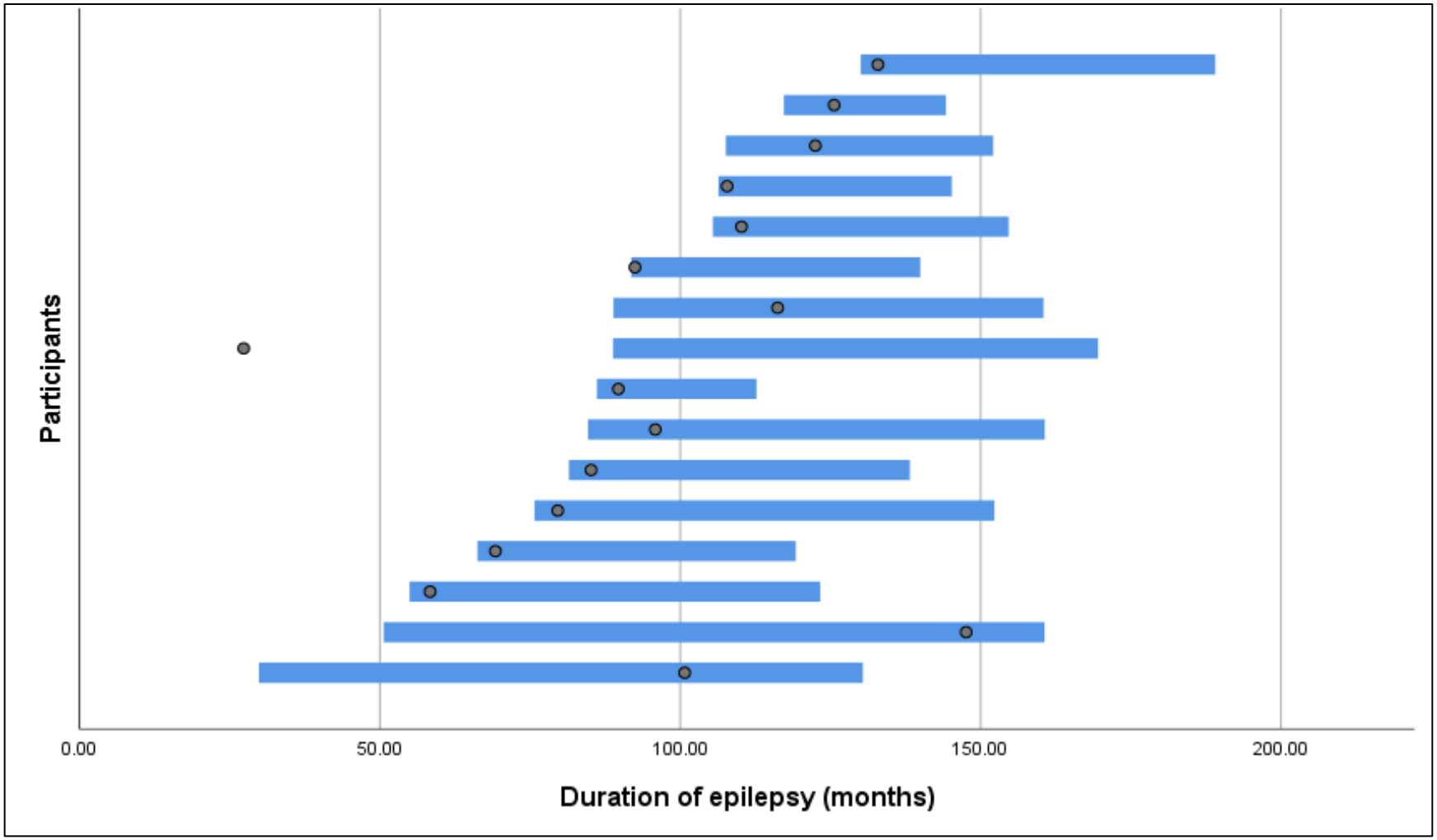


Figure 64: Duration of epilepsy in children with RE. Measured in months. The left edge of the blue bar denotes the first Rolandic seizure and the right edge of the bar denotes the last seizure. The grey circles denote the first documented evidence of RS on the EEG.

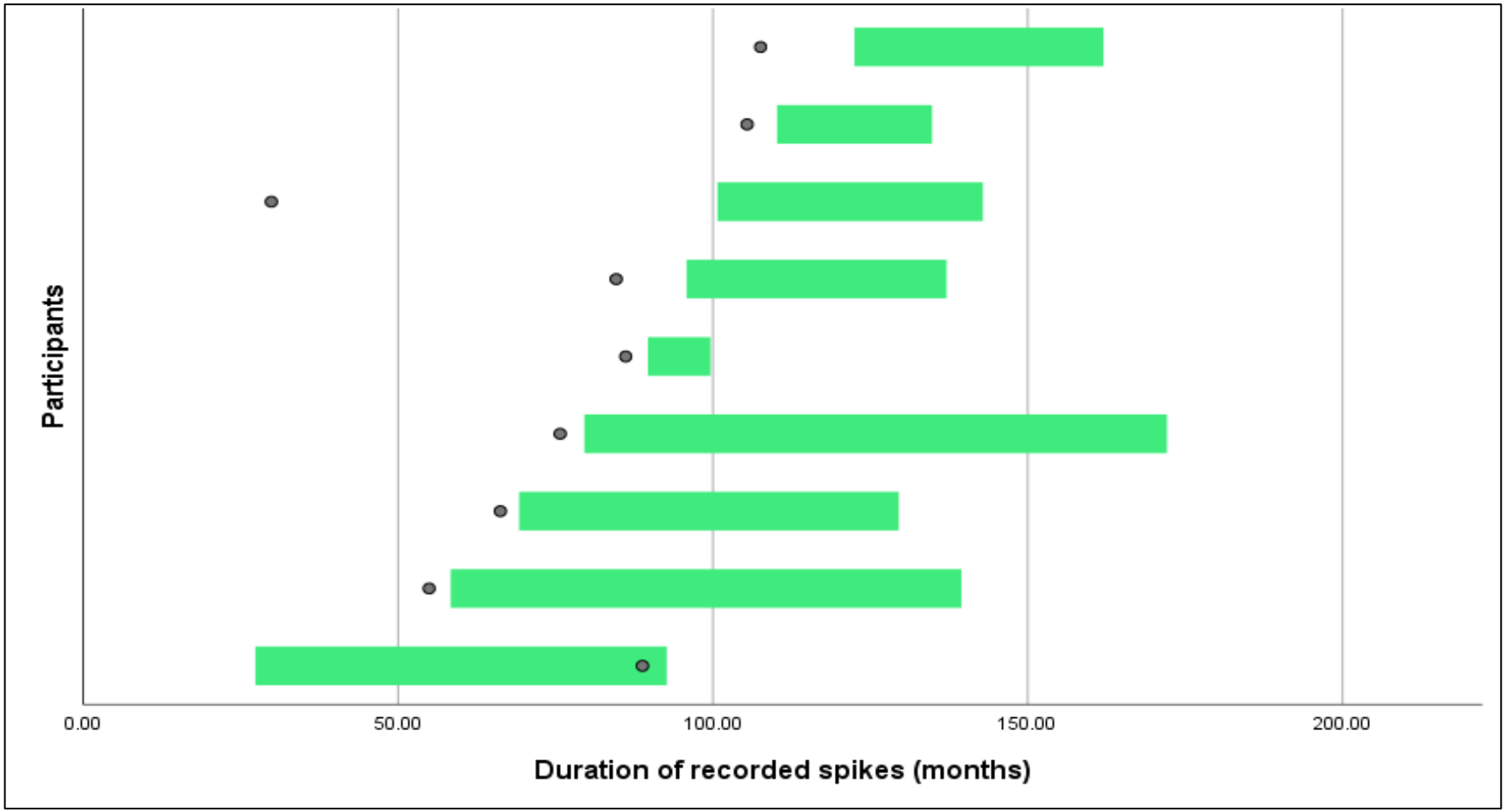


Figure 65: Duration of spikes present on scalp EEG. The left edge of green bar denotes when spikes were first recorded the right edge denotes when spikes were last seen on the EEG. The grey circle denotes the first Rolandic seizure. The number of participants in this group is restricted to those with more than one EEG.

The mean duration of epilepsy was 61.67±23.8 months, whereas the duration of recorded spikes was 72.51±27 months. Furthermore, as mentioned in one individual spike were seen 61.47 months before the generation of seizures, and in another, there was no evidence of spikes until 27.3 months into active epilepsy. A direct comparison between the duration of spikes and seizures cannot be made because the analysis was restricted by the number of EEG recordings.

In the initial diagnostic recording, fifty per cent of the participants attained a sleep state. 93.7% of participants had spikes in active epilepsy, only one participant had no detectable abnormalities, and this was recorded while the participant was awake. The majority had evidence of typical RS; these were predominantly bilateral with a right hemisphere emphasis. Indeed, most of the recordings demonstrated a right hemisphere abnormality (62.6%) (Figure 66).

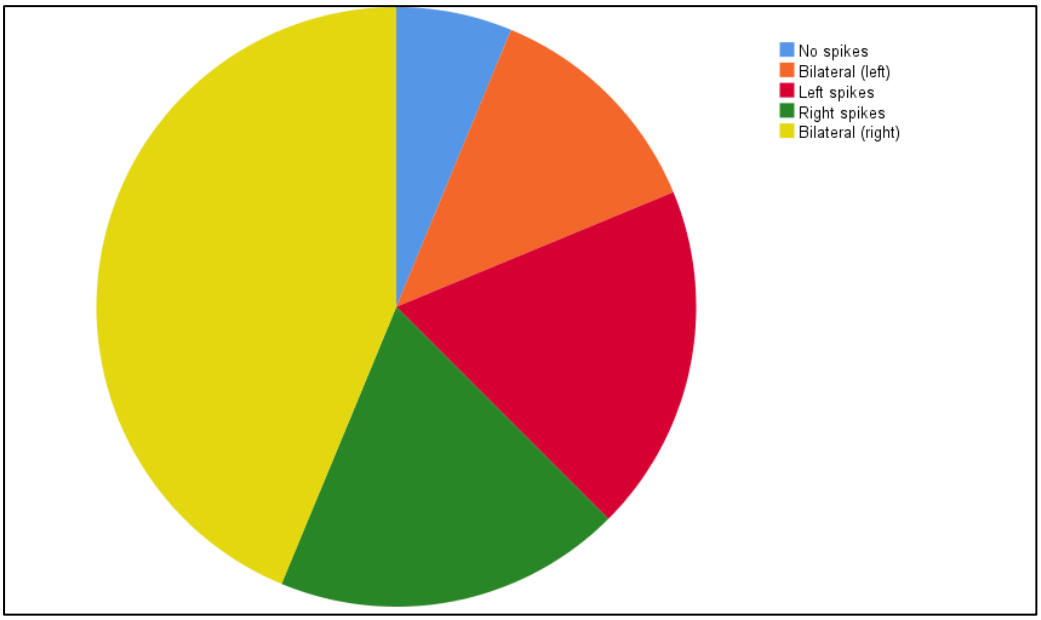


Figure 66: Proportions of spike hemisphere locations/distributions in the first recorded EEG. No detectable spikes (blue), left focal Rolandic spikes (red), right focal Rolandic spikes (green), bilateral Rolandic spikes with a left sided emphasis (orange), bilateral Rolandic spikes with a right-sided emphasis (yellow).

In those with multiple recordings, migration of spikes was the norm. Only two had fixed spikes (12.5%) before seizure remission. These included one participant with a fixed left and one with a fixed right hemisphere spike. These were seen in three separate EEG recordings and were seen in both an awake and sleep state.

7.3.1.2 *Follow-up EEG abnormalities*

Fourteen individuals returned for follow-up EEG; the duration of their seizure remission varied between 4-72 months with a mean of 22.35±18.34 months. Despite being in seizure remission, five of the participants were still receiving AED therapy. Three were on monotherapies of either levetiracetam, oxcarbazepine or sodium valproate. Two were on dual therapy of levetiracetam with either additional clobazam or perampanel. Eleven individuals were medication-free, only one had previously been on AED therapy (carbamazepine), and this was stopped 1.5 years before the recording.

Twelve participants entered a sleep state during follow-up EEG. Sleep was identified by the attenuation of the alpha rhythm and the appearance of sleep features such as vertex sharp waves, sleep spindles, K-complexes and positive occipital sharp transients of sleep which are indicative of non-rapid eye movement sleep 2 (NREM2) (Iber *et al.*, 2007). Two individuals were restless and did not enter a sleep state.

At follow-up, all participants had normal EEG backgrounds; however, epileptiform abnormalities persisted in 50% of the cohort, and this was regardless of AED use. Two participants on AEDs with a normal follow-up EEG were prescribed levetiracetam with either perampanel or clobazam. 28.6% of participants had focal spike/sharp epileptiform abnormalities in seizure remission; this consisted of four participants (Figure 67). Focal RS were seen in two participants over the left hemisphere, one participant on the right and one participant in a bilateral distribution. Two had their spikes while awake, and the rest had them in a sleep state. The spikes locations/distributions seen at follow-up were different from those recorded during active epilepsy. The left-sided spikes at follow-up were previously in a bilateral left and right distributions. The right-sided spikes at follow-up were previously on the left, and a previous bilateral with a right-sided emphasis was a bilateral distribution with no side emphasis at follow-up.

In addition to the focal spikes, the appearance of a new abnormality, generalised spike and wave discharges (GSW) were seen in six participants (42.9%). This abnormality is described as new because there was no mention of these discharges in previous clinical EEG reports, and this feature

was not apparent in those individuals where baseline EEG data had been reviewed by the researcher. Two of the participants with GSW had additional focal spikes located in the left and right hemispheres, respectively. Similar to the focal spikes, the discharges could be seen in an awake and sleep state, they were all similar in duration, around one second and the majority were associated with an alteration of the background EEG after the discharge. There appeared to be no clinical correlate associated with the discharges.

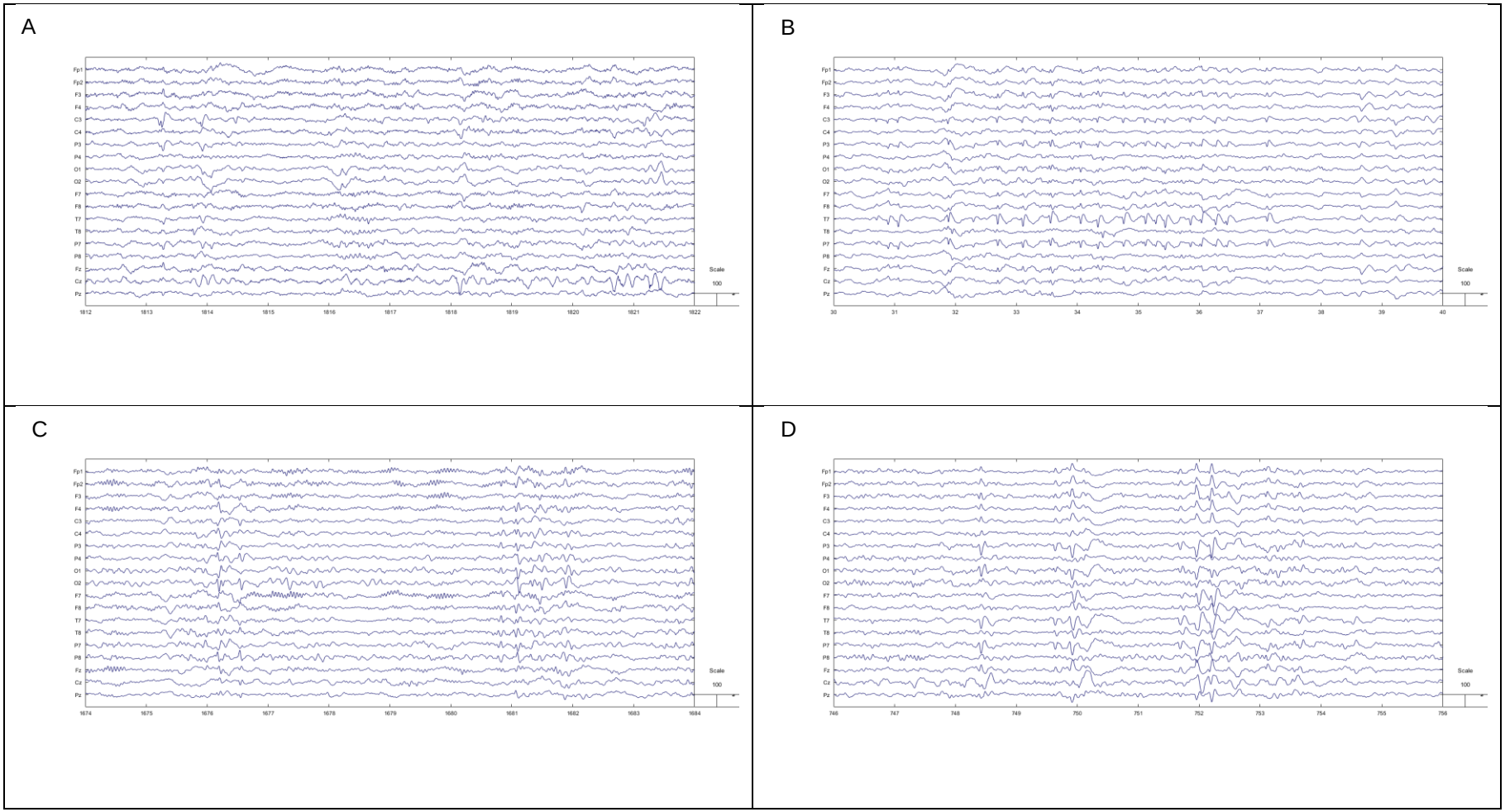


Figure 67: Evidence of Rolandic spikes in adolescents with Rolandic epilepsy in seizure remission. Sleep EEG recordings obtained at follow-up; each 10 second EEG extract contains evidence of focal spikes. Average reference EEG at a sampling rate of 256 Hz, LFF: 0.5 Hz, HF 70 Hz. Each EEG has a sensitivity bar of 100 µV per unit. A: Male, 13.5 years old and 9.95 months in seizure remission with discrete left centro-temporal spikes, B: Female, 10.79 years old and 10.28 months in seizure remission with florid left posterior temporal and parietal spikes. C: Male, 11.91 years old and 12.48 months in seizure remission with spikes which extend across with right hemisphere with an occipito-parietal emphasis. D: Male, 14.34 years and 19.81 months in seizure remission with spikes in both an awake and sleep state.

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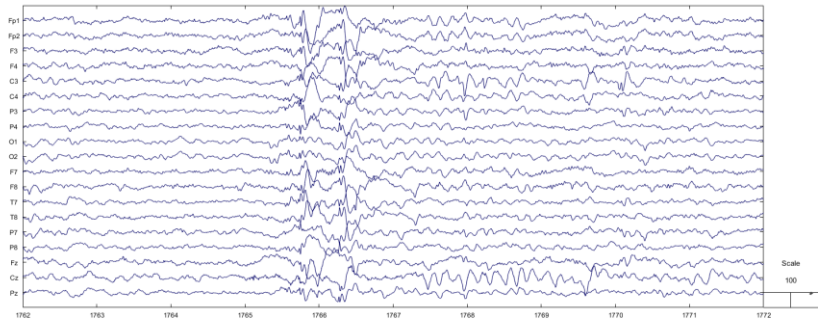
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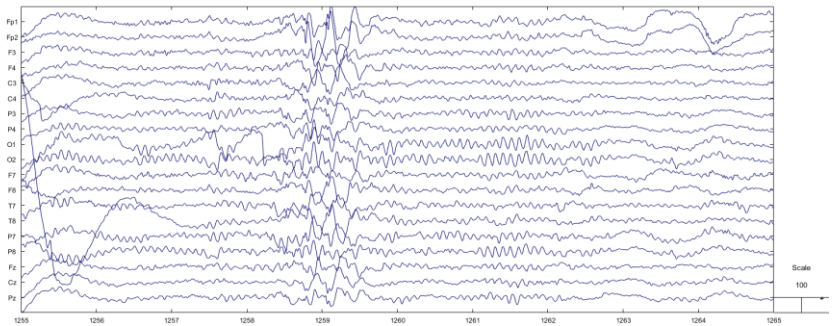
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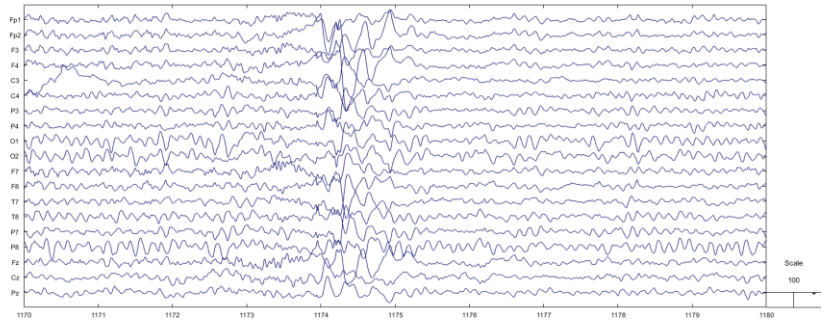
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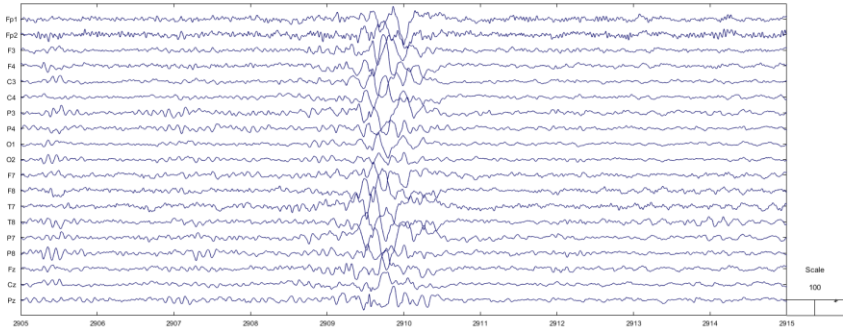
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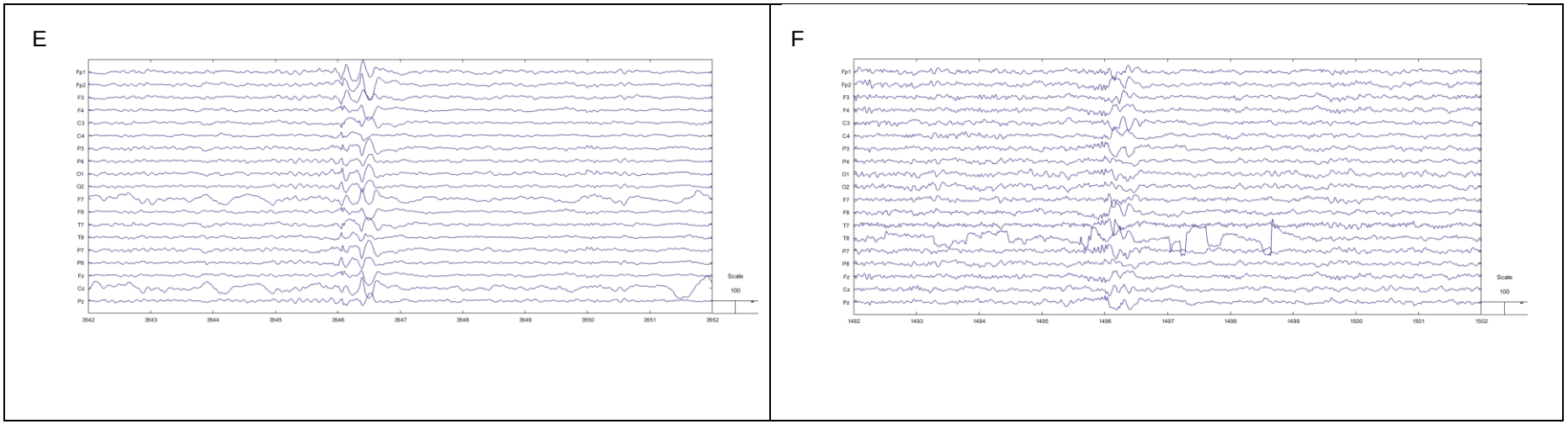


Figure 68: Evidence of generalised spikes and wave (GSW) discharges in adolescents with Rolandic epilepsy in seizure remission.

EEG recordings obtained at follow-up, each 10 second EEG extract contains evidence of focal, generalised spike and wave. Average reference EEG at a sampling rate 256 Hz, LFF: 0.5 Hz, HF 70 Hz. Each EEG has a sensitivity bar of 100 µV per unit. A: Male, 13.5 years old and 9.95 months in seizure remission. B: Male, 11.1 years old and 20.6 months in seizure remission. C: Male, 11.9 years old and 12.48 months in remission. D: Female, 14.21 years and 30.5 months in seizure remission. E: Male, 13.26 years old and 4.53 months in seizure remission. F: Female, 13.7 years old and 4 months in seizure remission. Note an artefact on the T8 (T4 10-20) These extracts show GSW discharges that can be seen in an awake and sleep state. They a brief in duration, around one second and consist of delta and theta activities with intermixed small sharp spikes. A, C and D are preceded or intermixed with fast activities. Interestingly, except for B there is a change in background before and after the discharge. This is either an attenuation or a change to the background frequency.

The age of the individuals in seizure remission, their epilepsy-related statistics and features in follow-up EEG are presented in Table 70.

EEG feature	NAD (n=7)	Abx (n = 7)	RS (n=2)	GSW (n= 3)	RS and GSW (n=2)	DF	Stats: NAD vs Abx (p=)
Age (years)	15.64±1.70	12.73±1.46	12.57±2.51	12.86±1.59	12.71±1.12	13	**0.005** [a]
Age range (years)	13.7-19.07	10.79-14.34	10.79-14.34	11.10-14.21	11.91-13.50	N/A	N/A
Epilepsy onset (years)	7.16±2.52	6.78±2.08	5.91±0.6	7.76±0.71	5.73±4.58	13	0.769
AED (%)	42.9	42.9	50	33.33	50	N/A	N/A
Duration (months)	72.18±23.7	57±24.21	64.79±16.68	41.29±12.79	72.53±39.6	13	0.308
Seizure remission (months)	33.45±22.39	14.02±9.05	15.05±6.74	14.91±12.96	11.21±1.78	13	**0.044**

Table 70: Demographics of EEG abnormalities in adolescents with Rolandic epilepsy in seizure remission.Age: Mean age is at the time of the follow-up EEG recording. Age range: Age range at follow-up. Epilepsy onset: Age of first seizure. AED: Percentage of participants on AED therapy, Duration: Duration of epilepsy. Seizure remission: Duration of seizure remission. Stats: A t-test compared normal versus abnormal EEG. [a] survived Bonferroni correction for multiple comparisons. DF: Degrees of freedom. NAD: No abnormalities detected, Abx: Abnormal EEG, RS: Rolandic spike: GSW: Generalised spike and wave.

At follow-up, a normal EEG was recorded in six participants; this group contained the oldest participants and had the longest period of seizure remission (Table 70). There was no evidence of abnormalities over the age of 15 years. A t-test revealed a significant difference ($p = 0.005$) in age between those with abnormalities on the EEG and those with a normal EEG and this survived Bonferroni correction for multiple comparisons. The duration of seizure remission was also significant, but this did not survive Bonferroni correction. The age of first seizure and the duration of epilepsy had no relationship with the appearance of abnormalities in the follow-up.

7.3.2 **Quantitative Analysis**

7.3.2.1 Demographics

A reduced sample was assessed due to limited access to retrospective clinical EEG recordings during active epilepsy. Baseline clinical EEG raw data were obtained for eight participants with active epilepsy. At follow-up in seizure remission, fourteen participants had non–clinical EEG recordings (Table 71). Unfortunately, one participant was removed from the active epilepsy group due to being 16 months into seizure remission at the time of the recording, and another was removed due to EEG artefacts. In the follow-up data, due to technical problems, one participant had to be removed from the analysis as the resting state could not be identified. Five participants had EEG recordings at both baseline and follow-up.

Time-point	Age (years)	Sex (%)	Handedness (%)	Onset (years)	AED (%)
Baseline (n=6)	8.71±1.83	83.3	83.3	7.11±2.24	33.3
Follow-up (n=13)	14.45±1.99	69.2	92.3	7.05±2.24	46.2

Table 71: Demographics of participants with EEG recordings in for quantitative analysis. Age: Age at recording. Sex: Percentage male, Handedness: Percentage righthanded, Onset: Age of first seizure. AED: Percentage taking an anti-epileptic drug.

7.3.2.2 *Global dominant frequency and EEG power density*

Global dominant frequency (GDF) increased, and the average total absolute power (TAP) decreased between active epilepsy and seizure remission. The dominant global rhythm increased by 1.03 Hz between active epilepsy and seizure remission and over the same period, total global power reduced by 173.71 μV²/Hz. These phenomena were both significant and had a large effect size (Table 72).

	Baseline (n=6)	Follow-up (n=13)	DF	Stat (p=)	Effect size (d)
GDF (Hz)	8.72±1.16	9.75±0.90	18	**0.050**	**0.99**
TAP (μV²/Hz)	418.33±244.46	244.62±117.67	18	**0.048**	**0.91**

Table 72: Global dominant frequency and total global power in active epilepsy and seizure remission. Global dominant frequency (GDF, Hz) and total absolute power (TAP, μV²/Hz). Stat: Unpaired t-test. DF: Degrees of freedom. Effect size: Cohens d. There is an increase in GDF and a decrease in total absolute power between baseline and follow-up.

A comparison was made for absolute and relative power for the five frequency bands. The absolute power of all frequency bands decreased between active epilepsy and seizure remission. The only significant change in absolute power was in the theta frequency band; however, this did not survive Bonferroni correction. A different and interesting change occurred in the relative power of frequency bands.

There was a decrease in delta and an increase in beta relative power between active epilepsy and seizure remission (Table 73). These changes were significant with large effect sizes and survived Bonferroni correction. The delta relative power decreased from 33.94 to 15.14% in effect halving, and the relative beta power increased from 37.16 to 56.49%.

Overall, absolute power decreased between active epilepsy and seizure remission, whereas changes in relative power were nuanced with a decrease in delta and an increase in beta prominent.

	Active epilepsy (n=6)		Seizure remission (n=13)						
	Absolute (μV²/Hz)	Relative (%)	Absolute (μV²/Hz)	Relative (%)	DF	Abs stat (p=)	Abs effect size (d)	Rel stat(p=)	Rel effect size (d)
Delta (1-3 Hz)	143.45±102.04	33.94±12.87	37.34±28.22	15.14±7.89	18	0.051	1.42	**0.0010** [a]	1.76
Theta (4-7 Hz)	61.04±32.41	15.25±2.25	36.00±17.93	15.10±3.59	18	**0.043**	0.96	0.92	0.05
Alpha (8-13 Hz)	46.97±37.65	10.38±2.26	23.39±14.78	9.68±3.77	18	0.19	0.82	0.68	0.23
Beta (14-30 Hz)	154.35±98.41	37.16±10.20	139.89±77.17	56.49±12.88	18	0.73	0.16	**0.005** [a]	1.66
Gamma (31-40 Hz)	13.00±9.75	3.26±1.90	8.05±4.42	3.58±1.79	18	0.28	0.65	0.73	0.17

Table 73: Absolute and relative power densities for each frequency band between active epilepsy and seizure remission.

Included are the absolute and relative power density values for delta, theta, alpha, beta and gamma frequencies. Abs: Absolute power (μV²/Hz) Rel: Relative power: Percentage of total power. Stat: The significance measured using an unpaired t-test. DF: Degrees of freedom. Effect size: Cohens D. Light blue shading indicates a decrease in power and red shading indicates an increase in power. [a] *survived Bonferroni correction across the five measurements.*

7.3.2.3 *Age and quantitative EEG measures*

To investigate whether these changes could be a result of normal development, these values were compared with the age of the participant when the EEG data was recorded. Linear regression found a significant relationship between GDF and age at the EEG (r= 0.457, p= 0.049), but this did not survive Bonferroni correction (Figure 69). Furthermore, analysis of total power found a significant relationship with age (r=0.472, p=0.041) (Figure 70). Pearson's correlation found a significant negative correlation between GDF and total power (-671, p=0.002), as GDF increases power decreases. When the relative power of frequency bands was compared to age, some strong relationships were revealed.

A decrease in relative delta and an increase in the relative beta were seen between active epilepsy and seizure remission (Figure 70). Linear regression found a significant trend for both delta (r= -0.766 p=0.00013) and beta relative power (r= 0.755, p = 0.00019) indicating a strong relationship between relative delta and beta power (Table 74). In summary, there is a strong relationship between GDF, total power and the relative power of the delta and beta frequency bands. To investigate whether this was influenced by age, a separate analysis investigated these values with respect to time to final seizure in both active epilepsy and seizure remission cohorts.

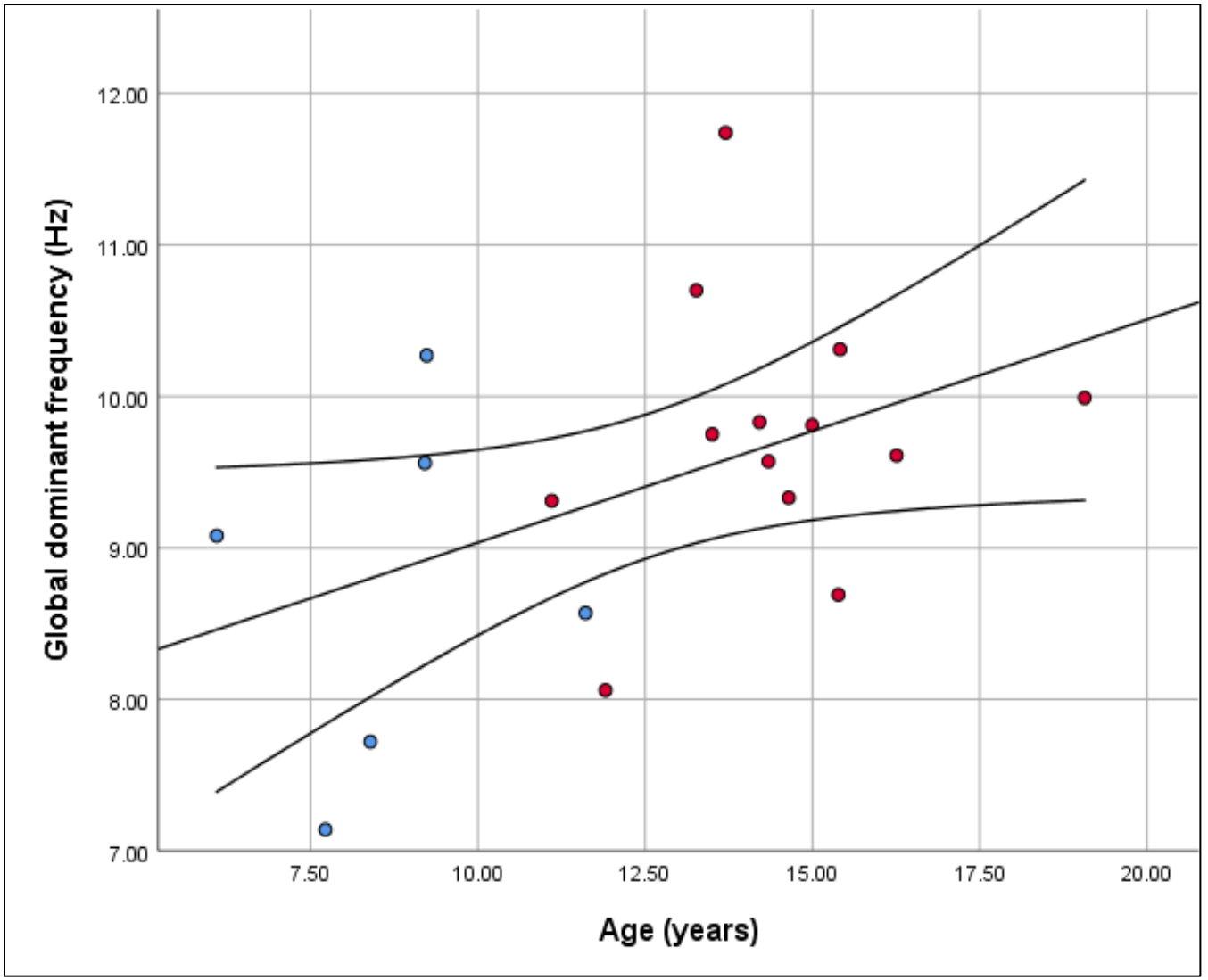


Figure 69: Global dominant EEG frequency and age in individuals with Rolandic epilepsy.

Age in years when the EEG was recorded. Regression line with 95% confidence interval. Blue dots active epilepsy and red dots seizure remission. There is moderate association between the global dominant frequency and age of the participant.

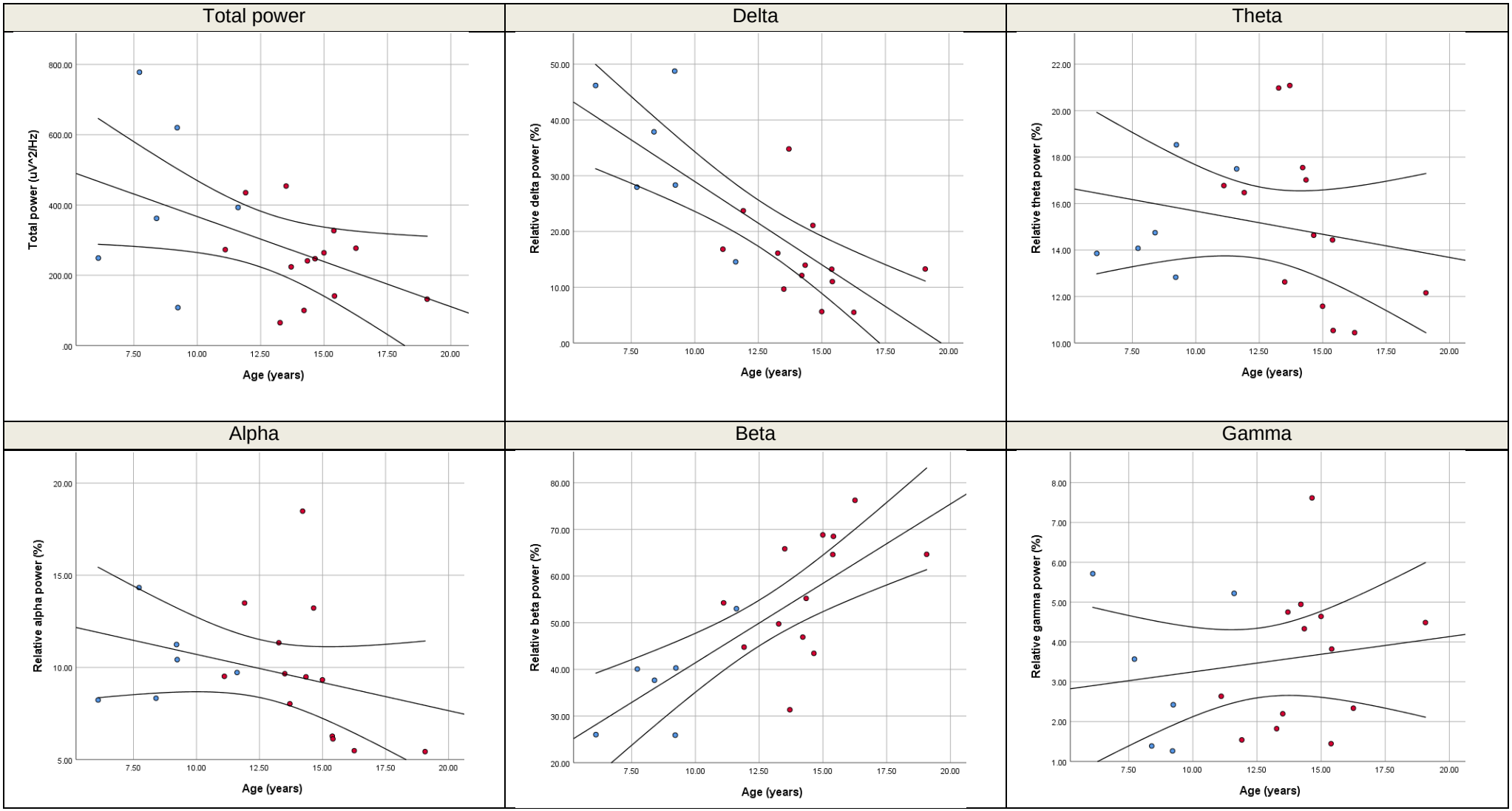


Figure 70: Changes in power density compared to age in individuals with Rolandic epilepsy.

The top left graph is total power measured in μV²/Hz. Clockwise from the middle top panel, relative power for delta, theta, gamma, beta and alpha frequency bands (% of total power). Blue dots: Active epilepsy. Red dots: Seizure remission. Relative delta and beta have a strong association with the age of the participant.

7.3.2.4 *Time from EEG to final seizure and quantitative EEG measures*

To explore the effect of seizure remission, the same data were plotted with respect to the time to final seizure; this was done in an attempt to remove the effects of age. The relationship between GDF and months to final seizure was non-significant (r=0.387, p=0.112) (Table 74,Figure 72). Linear regression results for time to final seizure revealed two significant results (Table 74).

	Age (r)	Stat (p=)	Final seizure (r)	Stat (p=)
Global dominant frequency	0.457	**0.049**	0.387	0.11
Delta relative power density	-0.766	**0.00013** [a]	-0.764	**0.00022** [a]
Theta relative power density	-0.209	0.39	-0.197	0.43
Alpha relative power density	-0.306	0.20	-0.323	0.19
Beta relative power density	0.755	**0.00019** [a]	0.759	**0.00026** [a]
Gamma relative power density	0.166	0.5	0.091	0.72

Table 74: The relationship between EEG metrics, age and months to final seizure in individuals with Rolandic epilepsy. Analysed were the global dominant frequency (GDF), delta, theta, alpha, beta and gamma relative power densities. Values in bold are significant. [a] survived Bonferroni correction for the five measurements. The was an association between global dominant frequency and relative delta and beta frequencies and age. Only relative delta and beta frequencies were significantly associated with time to final seizure.

Linear regression revealed a significant relationship between months to final seizure and the relative power of delta and beta frequencies (Table 74). Relative delta (r=-0.764, p=0.00022) and beta power (r=0.759, p=0.00026) both had a significant relationship with the time to final seizure. Overall, there was no relationship between GDF and time to final seizure, whereas relative beta and delta power

was significant. To further explore the relationship between relative power, age and time to final seizure, a hierarchical regression model was used.

Two hierarchical regression models were created with respect to age at test and time to final seizure. A backward stepwise exploratory method was utilised, the predictors in the model were relative, delta, theta, alpha, beta and gamma power. The best predictor for age was relative delta power, which accounted for 76.6% of the variance (t = -4.9, p = 0.00013). Similarly, this was the same predictor for time to final seizure, delta accounted for 76.6% of the variance (t = -4.74, p = 0.00022). The resulting series of models did not include relative beta power suggesting a correlation with one of the other variables. Pearson correlation of the individual relative powers identified a strong correlation between relative delta and beta (corr= -0.92, p = 1.9601E-8). A smaller correlation was found for theta (corr = -0.46, p= 0.043) (Figure 71). In summary, linear regression and Pearson's correlation analysis has revealed a relationship between relative delta power, age and time to final seizure. This phenomenon is inversely related to relative beta power.

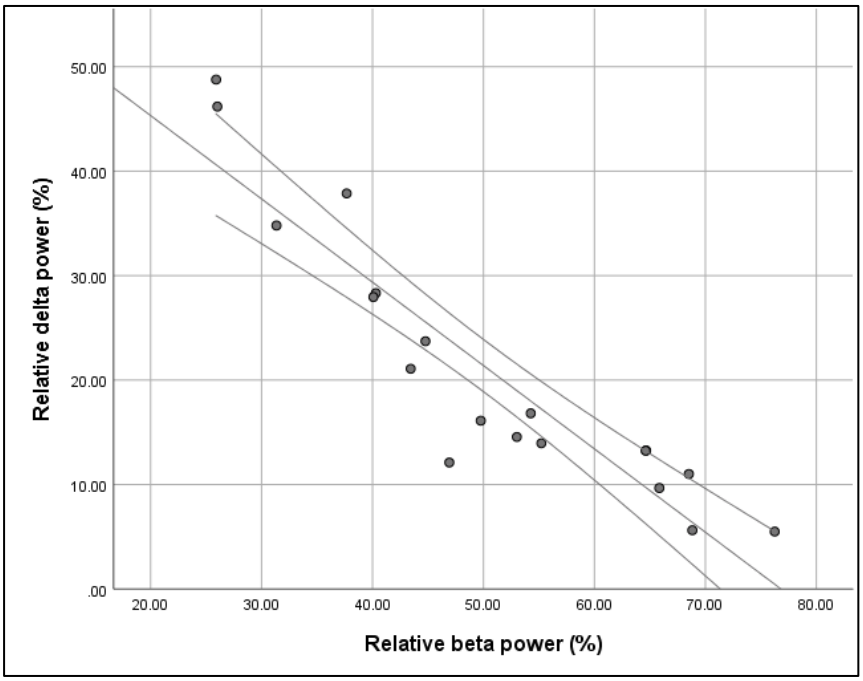


Figure 71: The correlation between relative delta and beta power in participants with RE. X axis beta power (%) and y axis delta power (%). The is a strong association between relative delta power and relative beta power.

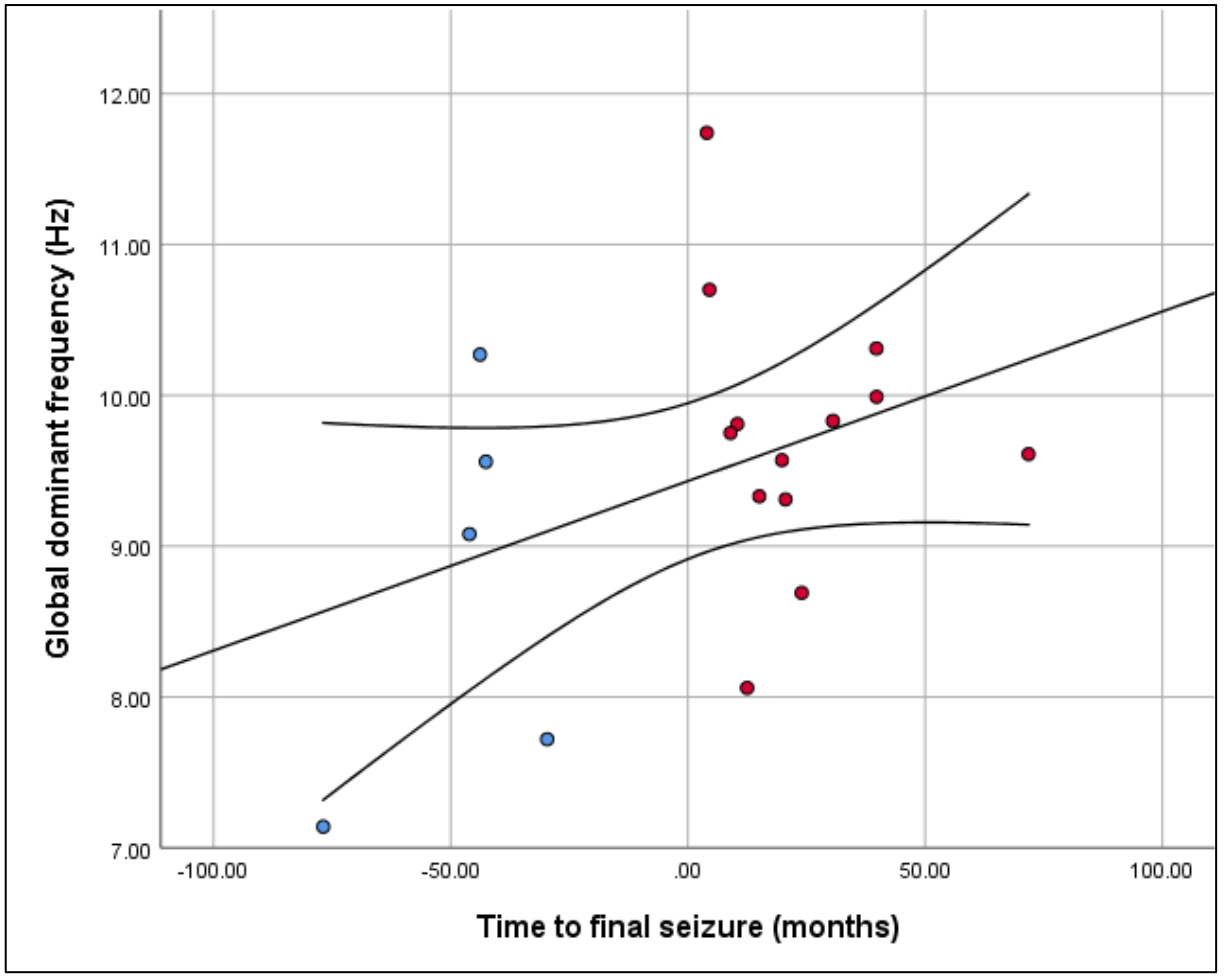


Figure 72: Global dominant frequency in individuals with Rolandic epilepsy before and after their final seizure. Baseline recordings (blue) and follow-up (red). Point zero on x axis = final seizure. There is no relationship between the GDF and time to final seizure.

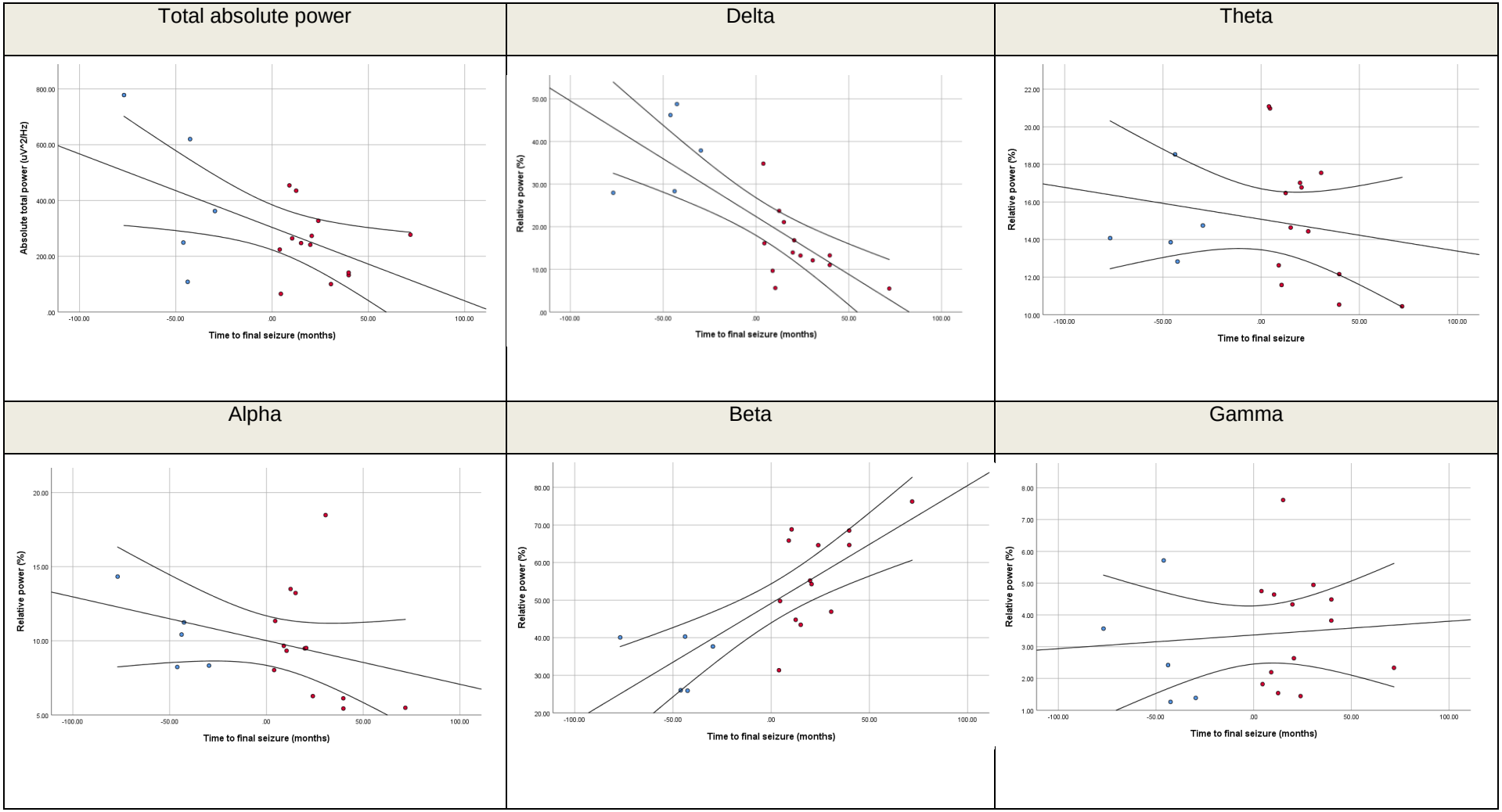


Figure 73: Relative power density of EEG frequencies in individuals with Rolandic epilepsy pre and post their final seizure.

Baseline EEG (blue) and follow-up EEG (red). Time of final seizure = Zero on the y-axis. Included are regression line and 95% confidence intervals. Total absolute power decreased over time to final seizure. Whereas the relative delta and beta power densities appeared to have a reciprocal relationship. As relative delta power decreased there was an increase relative beta power.

7.3.2.5 *Spectral changes between active epilepsy and seizure remission.*

Differences in absolute power can be seen between active epilepsy and seizure remission; these differences can be seen across all electrodes. Gamma power was significantly reduced (FDR corrected) between active epilepsy and seizure remission (Figure 74), this was seen across all of the electrodes with an emphasis over the frontal polar, midline and para-sagittal electrodes. One of the electrodes within the nineteen revealed another significantly different frequency power.

The mid-frontal electrode (Fz) revealed an additional significant difference in delta power Figure 74. The spectrum was enlarged (Figure 75) and specific frequencies with significantly altered power (FDR corrected) could be identified. In the delta frequencies the difference in power between active epilepsy and seizure remission was within 1-2 Hz whereas in the gamma frequencies this was between 34-40 Hz. In summary, the statistical analysis of resting-state absolute power spectra between active

epilepsy and seizure remission revealed a global decrease in specific gamma frequencies and a focal decrease in the mid-frontal regions.

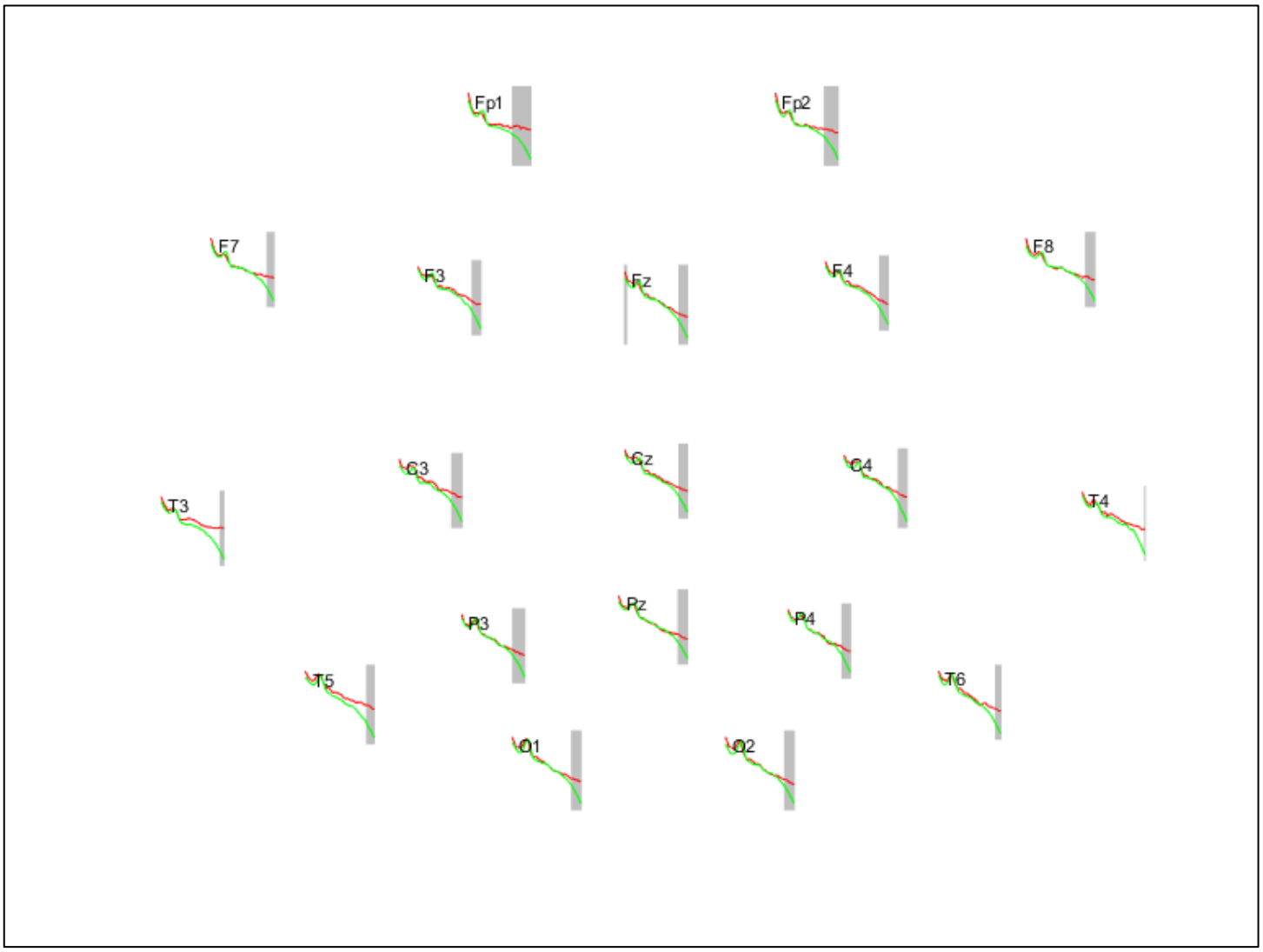


Figure 74 Average EEG spectra across electrodes between active epilepsy and seizure remission. The spectra's are between 0.5 and 45 Hz. Active epilepsy (red) and seizure remission (green). The grey shading represents a significant difference (p=<0.05) corrected for family-wise error using false discovery rate. All of the electrodes demonstrated a change in gamma between active epilepsy and seizure remission, this was greatest in the frontal electrodes. Only Fz demonstrates a significant change in delta frequencies between active epilepsy and seizure remission.

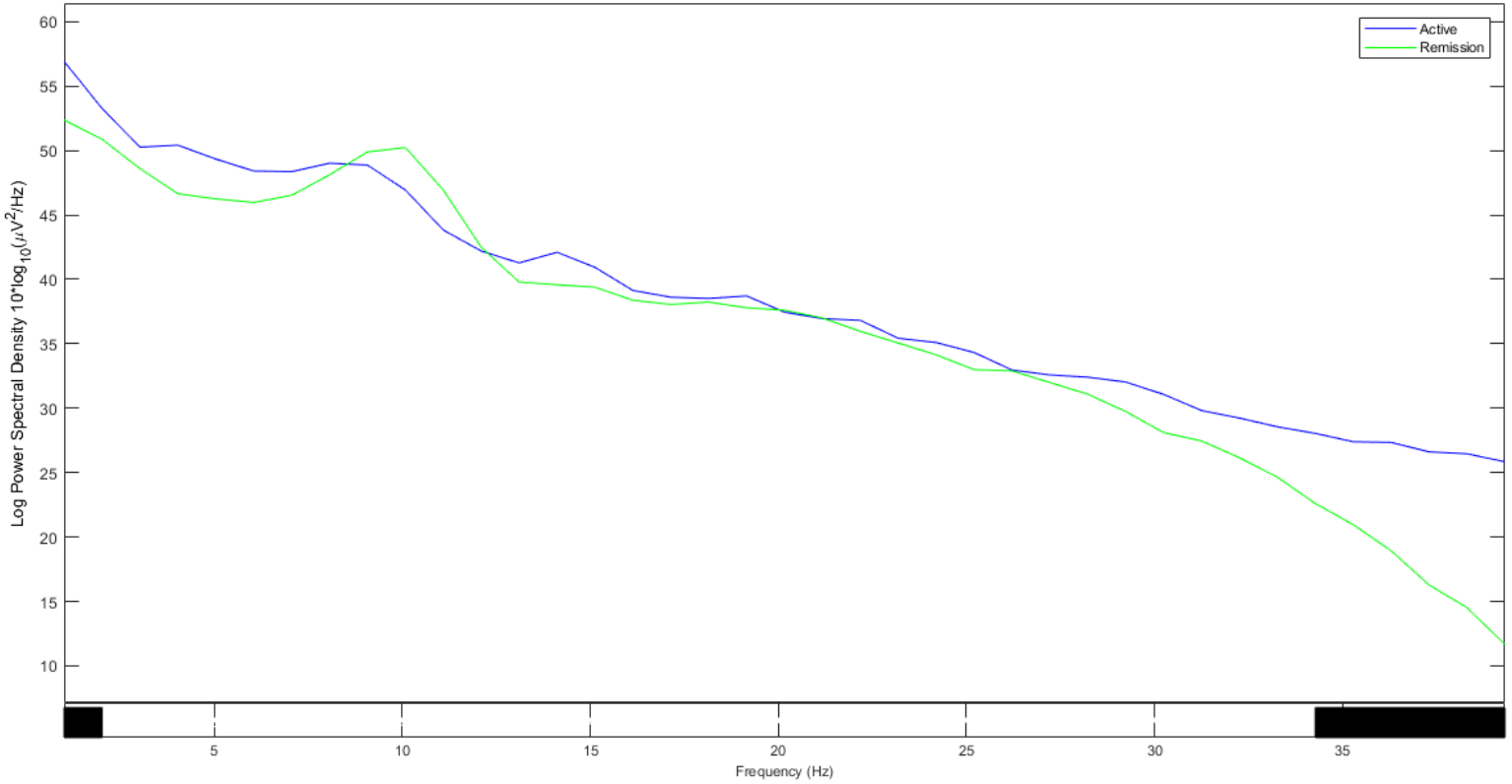
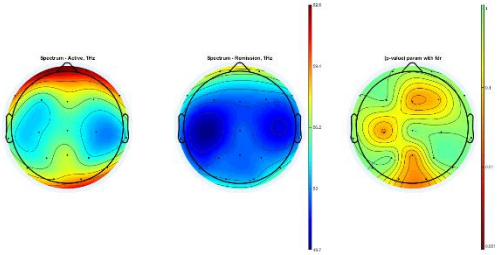
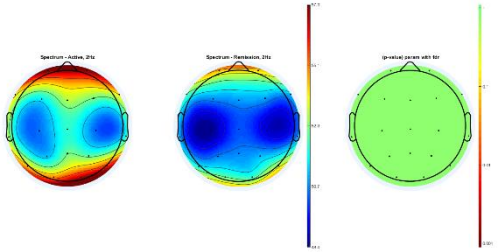
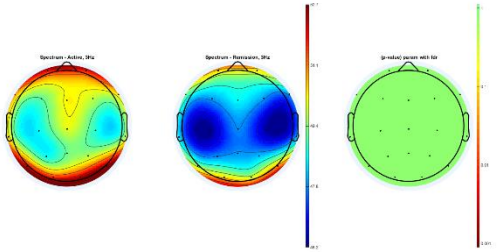
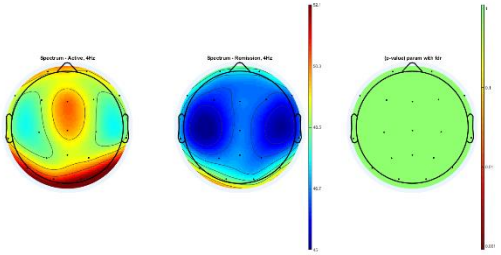
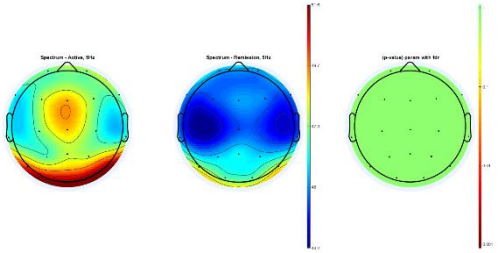
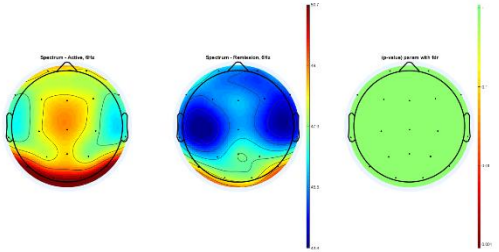
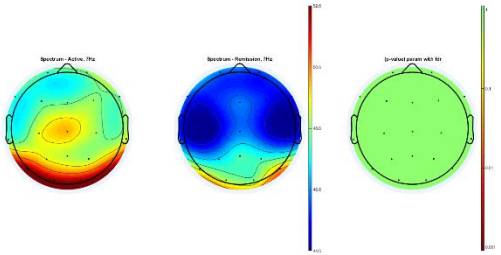
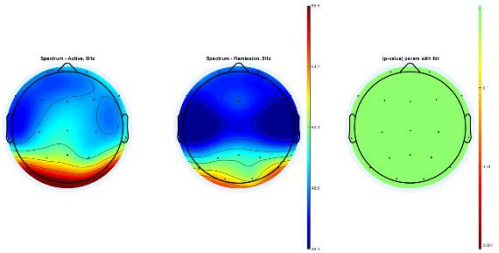
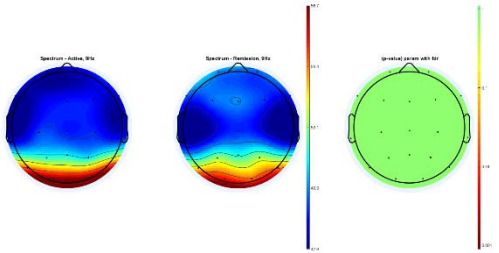
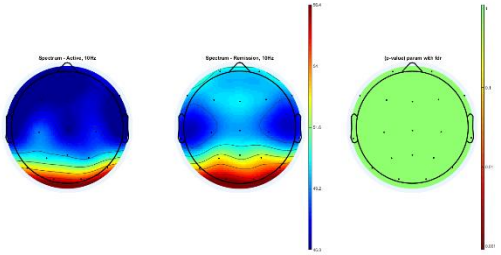
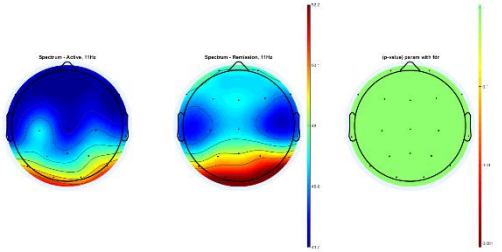
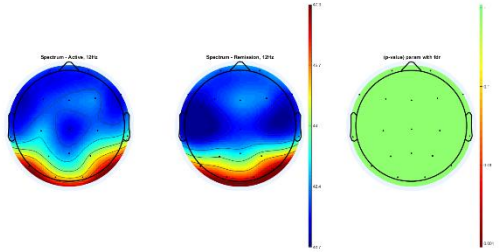
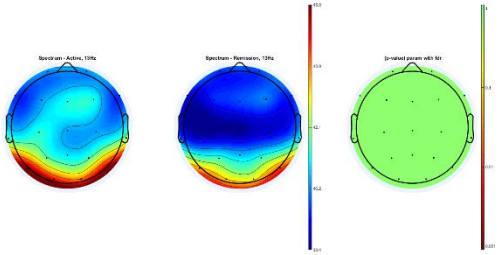
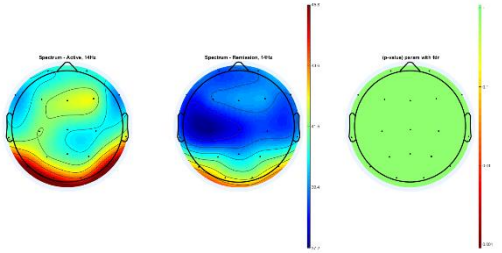
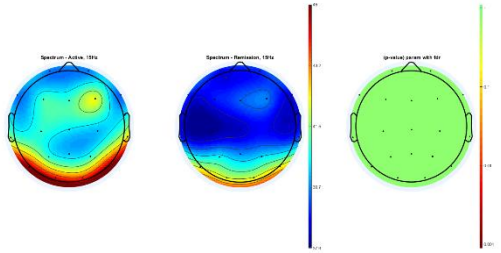


Figure 75: The EEG spectra at the Fz electrode between active epilepsy and seizure remission. This is an enlarged version of the spectra seen in Figure 74. Active epilepsy (blue) and seizure remission (green). The black bars underneath the X axis denote a significant difference between time-points (p=<0.05). Statistics are corrected for false discovery rate. The main points of interest are a reduction in delta power, a shift in the peak of the global dominant frequency and a drop in gamma power.

7.3.3 Topographic difference between baseline and seizure remission

1 Hz	2 Hz	3 Hz
		
4 Hz	5 Hz	6 Hz
		
7 Hz	8 Hz	9 Hz
		

10 Hz	11 Hz	12 Hz
		
13 Hz	14 Hz	15 Hz
		
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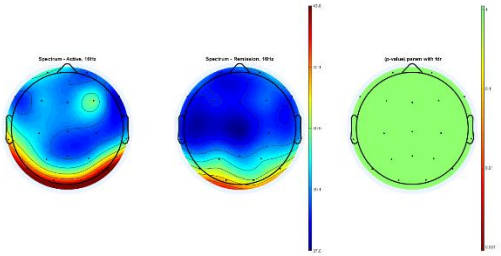	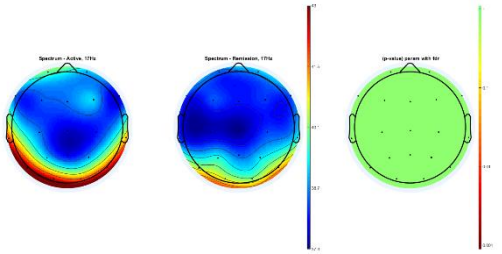	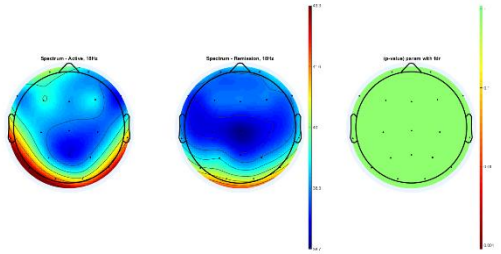

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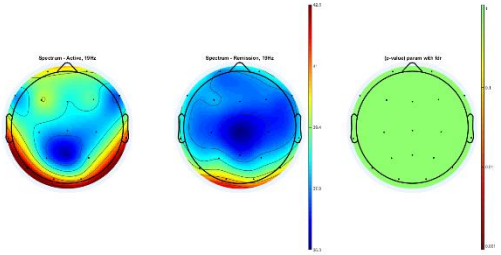	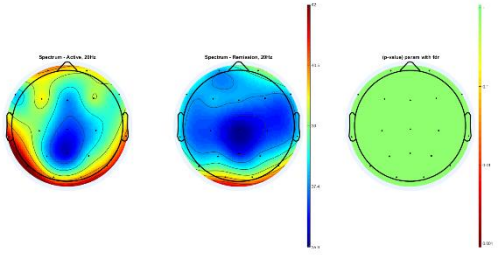	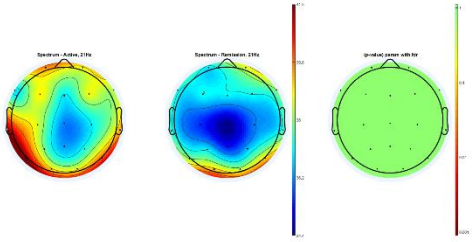
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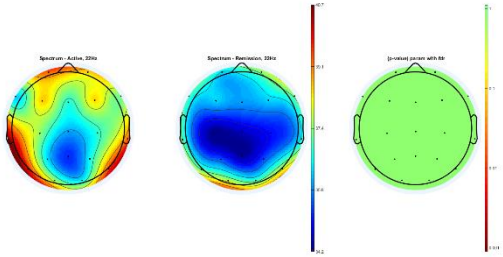
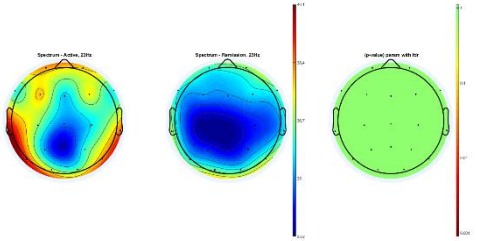
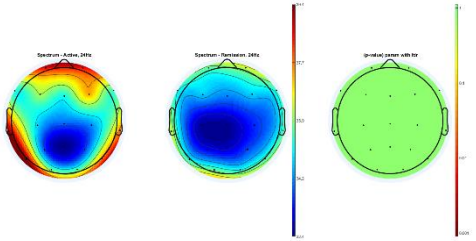
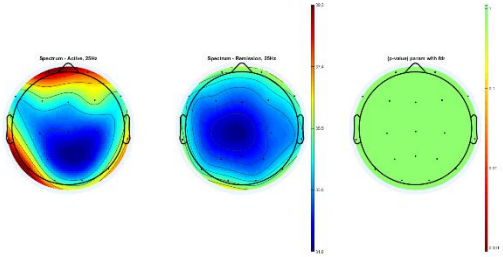
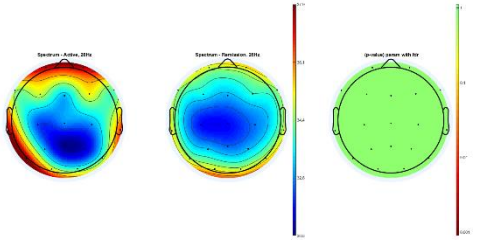
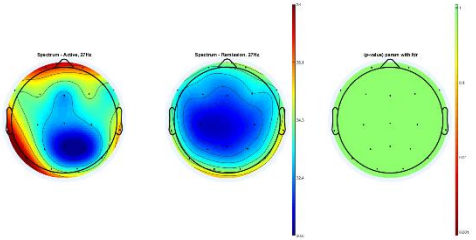
		
25 Hz	26 Hz	27 Hz
		

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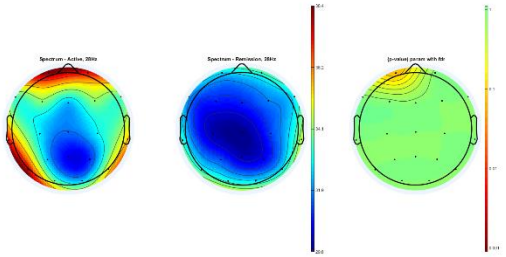
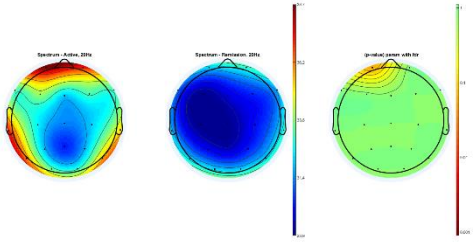
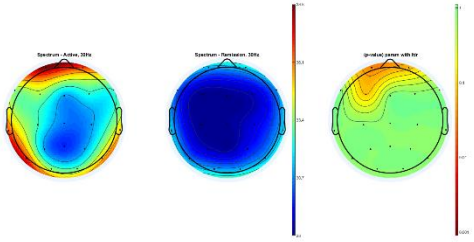
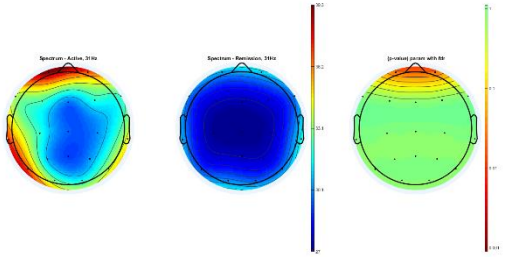
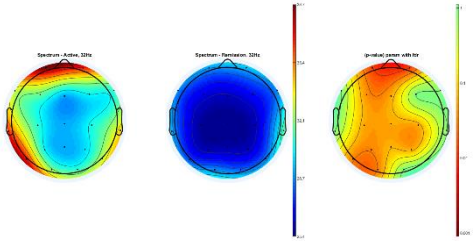
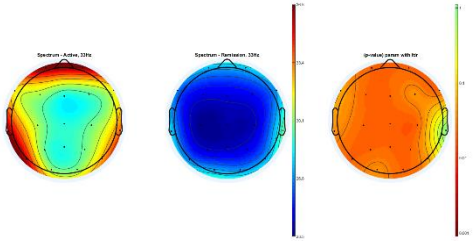
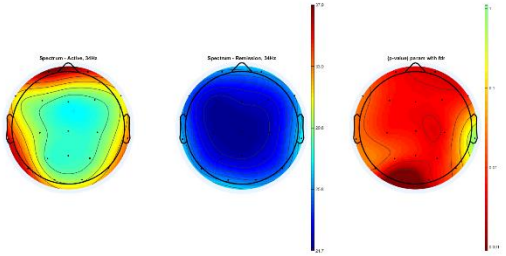
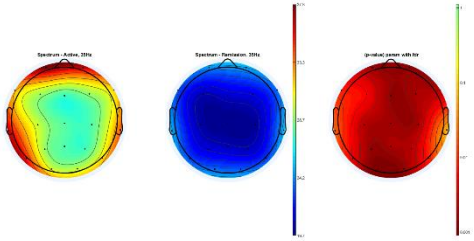
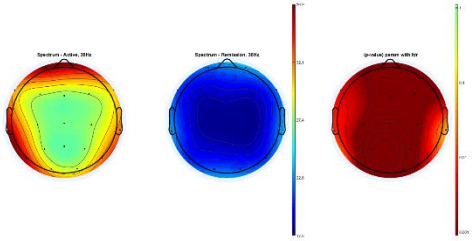
		
31 Hz	32 Hz	33 Hz
		
34 Hz	35 HZ	36 Hz
		

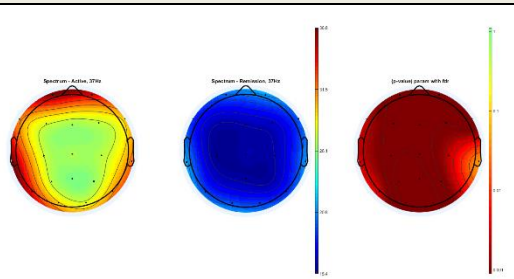
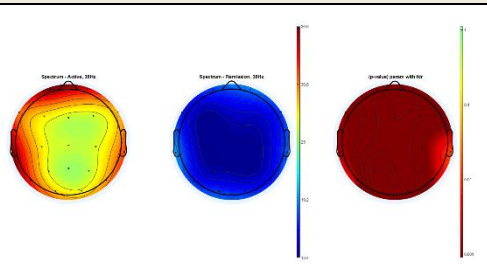
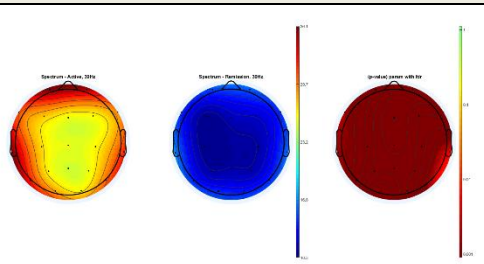
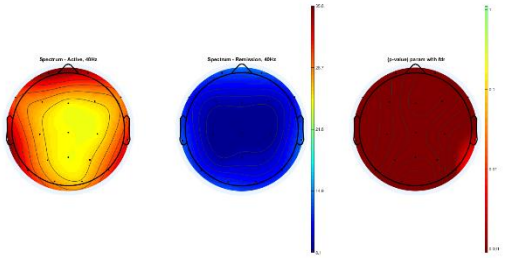
37 Hz	38 Hz	39 Hz
		
40 Hz		
		

Figure 76: Statistical analysis of the topographic spectrum with correction for multiple comparisons with a false discovery rate (FDR). From the left to right, scalp map in active epilepsy, scalp map in seizure remission and scalp map of p values. The top of the scalp map is anterior and bottom posterior. Electrode locations denoted by black dots. Frequencies spectrums range between 1 Hz to 40 Hz. The power and p-value scales for each diagram is included with each image.

To visualise the changes between active epilepsy and seizure remission (Figure 76), exploratory topographic scalp maps were created using interpolation of the electrodes. The frequency bands explored ranged between 1-40 Hz. These maps were statistically compared using an unpaired t-test and corrected for FDR. The t-test was unpaired as some of the participants did not have baseline or follow-up data. Similar to the electrode analysis; there were significant changes in delta and gamma power.

Altered delta frequencies were seen across the midline (Figure 76). At 1Hz, there were three regions which significantly different between active epilepsy and seizure remission. The first region was focussed over Fz with spread into right frontal regions, the second was directly over the C3 electrode, and the final region was triangular, the peak of the triangle was at the Pz electrode, and the triangle spanned out towards the O1 and O2 electrodes. The changes in gamma frequencies between active epilepsy and seizure remission were more extensive.

There were large changes in gamma power, which were both focal and generalised in distribution (Figure 76). These changes ranged between 28-40 Hz; the changes are very diffuse after 33 Hz the difference at frequencies 28 to 33 will be described. At 28 and 29 Hz, there is a small region of difference over the Fp1 electrode, at 30 and 31 Hz the difference in power remains localised within the bi-frontal regions. At 32 Hz the change in power spreads across the scalp with emphasis over the bifrontal regions, left posterior temporal and posterior to the right central electrode. At 33Hz most of the scalp has a significant change in this gamma frequency, leaving a small region in the right posterior temporal region which has lower p-value.

In summary, topographical analysis of interpolated EEG frequency absolute power reveals focal changes between active epilepsy and seizure remission in 1 Hz delta frequencies and variation of focal and diffuse difference in gamma frequencies.

7.4 Conclusions

7.4.1 Overview

This chapter aimed to describe and analyse brain function recorded by scalp EEG in individuals with RE between active epilepsy and seizure remission. A variety of methods and analyses have been utilised to explore changes. The main qualitative findings were, the majority of participants had spikes in active epilepsy, which were predominantly over the right hemisphere. One individual did not have focal spikes during active epilepsy, and another demonstrated spikes before epilepsy. Migratory spikes appear to be the rule and fixed the exception, though it may be the case that those with repeated EEG recordings were due to complications in seizure management. EEG abnormalities were not restricted to active epilepsy.

In seizure remission, the qualitative assessment found EEG abnormalities. Focal spikes can persist, and generalised spike and wave discharges can become apparent which were previously unreported in preceding EEGs. Younger adolescents and those with limited seizure remission were more likely to have these abnormalities. The focal abnormalities had migrated from the locations seen previous recordings. The quantified analysis also found differences between active epilepsy and seizure remission.

The quantitative findings demonstrate a large reduction in average relative delta power, which is associated with an increase in relative beta power. There is a large correlation between these phenomena and logistical regression found strong associations with both age and time to final seizure. A shift in averaged global dominant frequency between active epilepsy and seizure remission only had a relationship with the age of the individual. Changes in delta and gamma were seen in the topographies of EEG power.

The topographical analysis revealed statistically significant changes in the absolute power of delta and fast gamma activities. These changes involved large decreases in power between active epilepsy and seizure remission. Changes in 1 Hz delta power were localised to right frontal, left central and

bilateral occipital regions. Changes in gamma ≥28 Hz were frontal between 28-32 Hz and then became generalised and diffuse.

7.4.2 **Qualitative**

7.4.2.1 *The presence or absence of Rolandic spikes has a poor association with the susceptibility to Rolandic seizures.*

Rolandic spikes are apparent in most children with RE in active epilepsy; however, this study has evidence to suggest that RS, the relationship between spikes and seizures is weak. In this study, spikes could be recorded between 2 years old pre-epilepsy and up until the age of 14 years in seizure remission, whereas seizures were recorded between 2-11 years of age. The recording of spikes at the age of 2 years, raises the possibility that spikes could be apparent in children with RE before the generation of their first seizure, which thus weakens the role of RS in the generation of seizures. It would also suggest that there is a potentially long delay from the recording of spikes to the manifestation of the first seizure. Conversely, seizures can manifest without any evidence of RS on the EEG.

A meta-analysis by Bouma *et al.* found that 73% of patients had spikes apparent at the onset of epilepsy, whereas this study found 93.7% had spikes on their first EEG. The disparity between these two studies is most likely due to sample size and the ineffective methodology of obtaining hospital EEG reports. Nevertheless, the follow-up EEG recordings were a robust methodology and replicated findings reported in the literature.

The follow-up EEGs recording identified RS in 28.6% of the cohort in seizure remission. In the Bouma et al. meta-analysis, they report RS in 10.8% of those in seizure remission (Bouma *et al.*, 1997). There is a small difference in these findings, which may have been due to the type of EEG recording used. In this study, a prolonged sleep-deprived EEG was used, and there is evidence to suggest that increasing the duration of the EEG with sleep deprivation can increase the ability to capture EEG abnormalities (Reardon *et al.*, 1999). Interestingly there, appeared to be a cut-off age for the cessation of RS and this was found to be similar to the literature.

A recent study found 90% spike normalisation by the age of 15 years (Kim *et al.*, 2018). This study did not find any spikes over the age of 14.5 years. Kim also found that there was a significant positive

regression between the age of spike normalisation and age of seizure onset which would suggest earlier spike remission in individuals with late-onset epilepsy (Kim *et al.*, 2018). This would imply that the course of spike generation is separate from the ability to have seizures, other studies also find no relationship between the presence (Mcnally and Kossoff, 2015) or density (Lerman and Kivity, 1975) of RS and seizures Overall, these findings would suggest an additive mechanism in conjunction with spikes for the generation of seizures.

7.4.2.2 *New abnormalities can be seen in seizure remission*

The appearance of previously unreported GSW was an unexpected finding, but it would represent a new form of hyperexcitability in seizure remission. New abnormalities were not expected as it is postulated that cortical hyper-excitability decreases as the child enters seizure remission. The two reasons for this was one; the RS is a feature of cortical hyperexcitability (Manganotti *et al.*, 1998b) and two the cessation of seizures is believed to be due to a reduction in cortical hyperexcitability (Pawley *et al.*, 2017). Therefore, a sensible hypothesis is that in seizure remission in RE the cortex would be less excitable, which would make the production of new abnormalities counterintuitive.

A possible explanation for the GSW could be that they are a new expression of the RS due to a change in cortical circuitry, such as improved connectivity between the hemispheres due to white matter maturation in adolescence (Luders, Thompson and Toga, 2010). Alteration in connectivity could be possible; however, it does not explain why some participants had both RS and GSW. Another interpretation is that there are patchy changes in hyper-excitability across the cortex, so a reduction in one region may lead to an electrophysiological imbalance and GSW are a result. Finally, it could be the case that the ability to generate GSW discharges has always been present, and in seizure remission due to the reduced density of RS, the capacity to generate them has increased. This final explanation may be the best as similar GSW phenomena been reported in children with RE and an absence of RS.

There is evidence to suggest that RS on the EEG is not required to produce Rolandic seizures. Vargas et al. found in a group of 13 children with seizures typical of RE whose sole EEG abnormalities were generalised spike and wave (GSW) paroxysms on EEG and no evidence of RS. These discharges had a voltage asymmetry with an anterior emphasis and were observed in both wakefulness and sleep (Vargas et al., 2018) (Figure 77). Four of the individuals subsequently generated focal spikes in their sleep EEG. Interestingly, 54% of the patients had persisting GSW discharges in early seizure remission (Vargas et al., 2018). This small cohort demonstrates that GSW can be the sole inter-ictal feature seen in children with RE, which can persist after seizure remission. GSW has been reported in other cohorts.

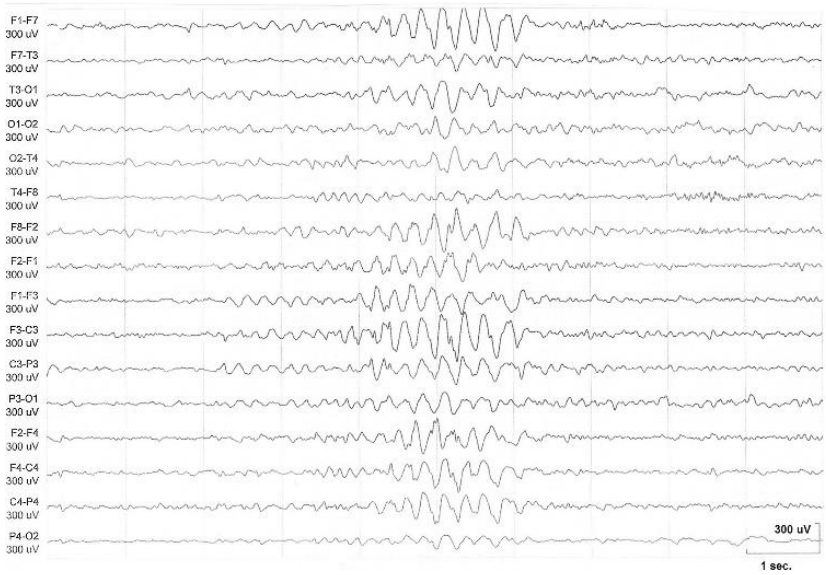


Figure 77: Generalised spike and wave in a 7-year-old boy with RE. EEG with a bipolar montage, sensitivity 300 µV per division. The GSW has a left frontal lead and there is a subtle attenuation and frequency change of the background EEG after the discharge. Extract from Vargas et al (2018).

A proportion of children with RE and RS have additional GSW. Studies report a wide range of prevalence of GSW in RE. Beaussart *et al.* found additional GSW in 6.8% (Beaussart, 1972), Beydoun *et al.* found 14.6% (Beydoun, Garofalo and Drury, 1992) and Beaumonoir *et al.* found 53.8% (Beaumanoir et al., 1974). It may be possible that GSW discharges are a feature of RE, but due to recording duration constraints and the appearance of the GSW is poorly formed that they are rarely identified or reported (Figure 77). Clinically, there is no reason to report a poorly formed generalised discharge when abundant RS would fulfil the electro-clinical diagnosis for RE (Koutroumanidis *et al.*, 2017). Other, idiopathic epilepsies of childhood demonstrate these features. The literature would suggest that GSW in RE are a common phenomenon; therefore, it is worth exploring whether this feature could play a role in ictogenesis.

There are several reasons why the GSW seen in these children may be a remnant of an ictogenic mechanism. One, the GSWs seen are unlike the generalised discharges seen in IGE. They are poorly formed, at times preceded by fast activity, and most are associated with either electro-decrement or frequency change after the discharges. These features of electro-decrement and fast activity have similarities which the onset of ictal EEG of children with RE. In a large study by Capovilla et al. they

found in 30 patients with RE with ictal recordings, 50% produced either low voltage fast or focal electro-decrement (Capovilla *et al.*, 2011). Smaller case studies found a similar abrupt cessation of inter-ictal spikes and low amplitude beta at the onset of a subclinical seizure (Bernardina and Tassinari, 1975; Gutierrez, Brick and Bodensteiner, 1990).

Two, the generalised discharges predominantly contain spikes, not RS sharp waves. Spikes on a paediatric EEG hold a greater weight towards supporting an epilepsy diagnosis compared to sharp waves (Smith, 2005). Moreover, there is a large body of evidence that spikes (≤70 ms duration) are the epileptiform feature which can alter brain function and have an ictal effect. Epileptic spikes are associated with myoclonic jerks (Wolf *et al.*, 2015), epilepsy with photo-sensitivity and eyelid flickering (Poleon and Szaflarski, 2017) and typical absence seizures (Crunelli and Leresche, 2002) There is scant evidence of sharp wave discharges having direct ictal manifestations such as transient cognitive impairment, sensory or motor manifestations.

Three, the discharges were seen during sleep deprivation, this is in keeping with the nature of RE where a predominant amount of seizures are elicited through poor sleep hygiene and subsequent sleep deprivation (Guerrini and Pellacani, 2012). It is important to note that sleep deprivation also evokes RS, but the previous statements do not rule out the involvement of RS in the generation of seizures, but it treats them as a secondary component within an ictogenic network. It is also important to explore the possibility that the GSW represents an EEG genetic marker or brain immaturity similar to the RS and is similarly not directly involved in the generation of seizures as it is routinely seen in other idiopathic focal epilepsies of childhood.

All idiopathic focal epilepsies of childhood have a proportion of children with GSW. Up to 23.7 % of children with Panayiotopoulos syndrome (Ohtsu *et al.*, 2003; Specchio *et al.*, 2010) will have GSW. The discharges described as “brief 1-3 s diffuse slow-wave complexes with usually small spikes” (Specchio *et al.*, 2010). Caraballo *et al.* found that 27% of children with the idiopathic occipital epilepsy of Gastaut produced GSW (Caraballo, Cersósimo and Fejerman, 2008). This evidence would suggest that GSW are features which can be seen in children with idiopathic focal epilepsies of childhood and could be features which are unique to all epilepsies within the benign seizure susceptibility syndromes (Panayiotopoulos *et al.*, 2008).

Overall, the appearance of GSW discharges in seizure remission would suggest that there is still the possibility of altered cortical function, and this may be due to altered brain development.

7.4.3 **Quantitative**

7.4.3.1 *Global dominant frequency increases in individuals with RE but this maturity is not related to seizure remission.*

There appears to be age-dependent maturation of the GDF, but this has no relationship with the remission of seizures. The group mean dominant global frequency significantly increased between active epilepsy and seizure remission from 8.72Hz to 9.75 Hz between the mean age of 8.7 years to 14.45 years, these findings are possible evidence of the maturity of the GDF. The GDF is a composition of dominant rhythms which includes mu rhythm and a large amount of alpha (Bazanova and Vernon, 2014). As GDF is rarely examined and as a proxy measure, it is similar to alpha in these conclusions' studies of alpha or peak alpha frequency will; be discussed. These studies have found similar evidence of an increase in alpha frequency between childhood and adolescence.

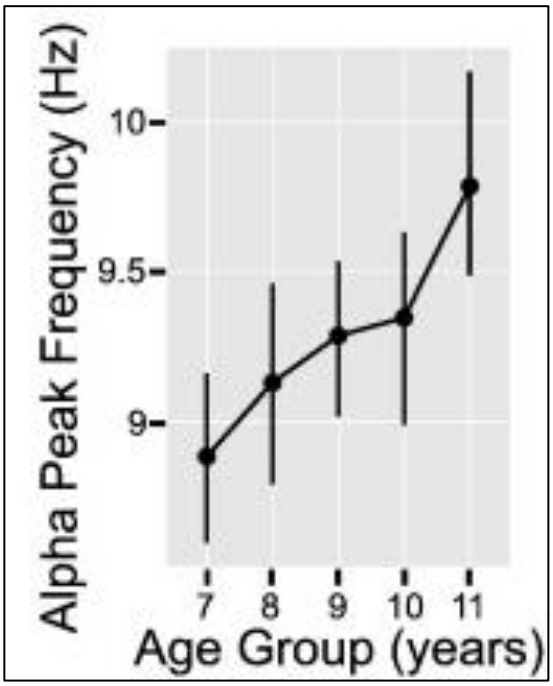


Figure 78: Mean alpha frequency across five ages groups between aged 7 to 11 years. Error bars: 95% confidence intervals estimated using bootstrapping with 1000 random iterations. Extracted and adapted from Miskovic et al (2015)

A cross-sectional study found a mean value of alpha peak frequency at 8.89 Hz in a group of 61 children aged seven years and 9.79 Hz in a group of 47 children aged eleven years (Miskovic *et al.*, 2015) (Figure 78). They found a linear increase in alpha between these age groups. Similar findings have been found in longitudinal studies, Cragg *et al.* found in 54 adolescents a significant increase in frequency with age between 10-13 years of age (Cragg *et al.*, 2011). This increase in the GDF is thought to reflect the development of connections between cortical and subcortical structures.

It is hypothesised that alpha activity is thought to reflect highly synchronous activity driven by thalamocortical interactions. (Whitford *et al.*, 2007). The increase in the frequency of the alpha rhythms is likely to be influenced by the development of the thalamus and thalamocortical connections in conjunction with synaptic pruning in the cortex. In particular, a recent study has evidence that the integrity of white matter connections. Valdés-Hernández al found that there was a significant relationship between the fractional anisotropy of the posterior commissural fibres of the corpus callosum (Valdés-Hernández *et al.*, 2010). These findings would suggest a normal development of the GDF however, without control data, this cannot be confirmed. Nevertheless, an interesting feature of the increase in the GDF is the lack of relationship with the time to final seizure. The lack of a relationship between PAF and final seizure could suggest that this maturational process is not required for seizure remission. As this is the first study to report this finding a comparison is difficult to make, therefore, it would be prudent to repeat the analysis with a larger data set.

7.4.3.2 *Global absolute power and relative delta power reduces in seizure remission*

Global EEG power decreased in seizure remission, and this seemed to be largely correlated with a reduction in relative delta power, this is in keeping with the literature of typically developing (TD) children. Whitford *et al.* found a reduction in global EEG power between healthy children, adolescents and adults (Whitford *et al.*, 2007). Similarly, Cragg *et al.,* found a decrease in absolute power with age, which was at its greatest in the delta and theta frequency range. Relative power changes in TD children involved increases in alpha and beta frequencies. It is hypothesised that spectral power decreases as a result of the pruning of synapses and the neuropil in the cortex (Whitford *et al.*, 2007). Indeed, Whitford et al., demonstrated in 138 participants between 10-30 years of age that absolute EEG power correlated with grey matter volume in the frontal and parietal regions (Whitford *et al.*, 2007). As grey matter volume decreased, delta power decreased. This data would suggest that the maturation of the EEG individuals with RE is following a normal developmental trajectory; it is unclear is an increase in relative beta power is part of this trajectory.

The findings of this study show a close relationship between the decrease in relative delta and an increase in relative beta, but this is not seen in other studies. Barriga-Paulino *et al.* calculated the mean power across all electrodes and found in a group of 8-23-year-olds that there was a non-significant negative correlation (R = -0.112) between relative beta and delta, suggesting that as relative delta power decreases there were minor increases in beta power. (Barriga-Paulino, Flores and Gómez, 2011). Other studies have found that it is theta rather than delta which have an inverse relationship with beta power, in particular, low beta 15-20 Hz in the maturation of EEG spectra (Rodríguez-Martínez *et al.*, 2015). Comparison with other studies would suggest that children with RE are following a normal developmental path, but the extent of changes and the inter-relationships between frequency bands may be atypical. Despite this, it would seem that a reduction in delta power is strongly associated with the remission of seizures.

Delta activity in the awake resting state EEG is evidence of neurological immaturity. Delta wave activity can be seen extensively in young children up to the age of 4 years (Clarke *et al.*, 2001). Crag *et al.* found relative delta activity at the age of 10, which decreased over three years; this was predominantly over the bilateral posterior regions (Cragg *et al.*, 2011). As previously mentioned, delta

power has been associated with grey matter volume in the frontal and parietal lobes (Whitford *et al.*, 2007). This would suggest that the reduction relative delta power is related to a reduction in cortical volume, which leads to seizure remission in individuals with RE.

7.4.3.3 *Topographic analysis reveals large charges in 1 Hz delta frequency absolute power between active epilepsy and seizure remission.*

The topographic analysis found significant changes in delta along the midline and left central regions. These changes are different from those identified by Crag *et al*. and would suggest that in RE, there are delta generators outside of the posterior regions. Excessive delta can be seen in the resting state of neurodevelopmental disorders which include attention deficit hyperactivity disorder (Barry, Clarke and Johnstone, 2003), dyslexia (Ackerman *et al.*, 1994) and autism (Chan, Sze and Cheung, 2007). As far as this author is aware, there are no published papers on the resting state of developmental coordination disorder or children with developmental language disorders. Despite this, Ackerman *et al.*, found no significant difference in delta power between groups with ADHD, dyslexia and slow learners (Ackerman *et al.*, 1994) which is evidence to suggest a continuum of excess delta in between apparently dissimilar neuro-developmental disorders. Overall, the possible increase in delta power would suggest that individuals with RE have similarities with individuals with neurodevelopmental disorders.

7.4.3.4 *Topographic analysis revealed large changes in gamma frequency absolute power between active epilepsy and seizure remission.*

The decrease in resting gamma is evidence of normal development. In a cross-sectional study by Tierney *et al.*, they found that gamma power decreased with age in 156 healthy controls, and this occurred in global distribution (Tierney *et al.*, 2013). Tierney noticed that this decrease was similar to changes in grey matter volume and absolute EEG power measured by Whitford *et al.* and hypothesised that the changes in resting gamma might reflect decreases in synaptic density linked to synaptic pruning (Whitford *et al.*, 2007; Tierney *et al.*, 2013). The question remains whether these changes reflect a cortical source, the result of contamination by artefacts such as electromyographic activity or other developmental changes.

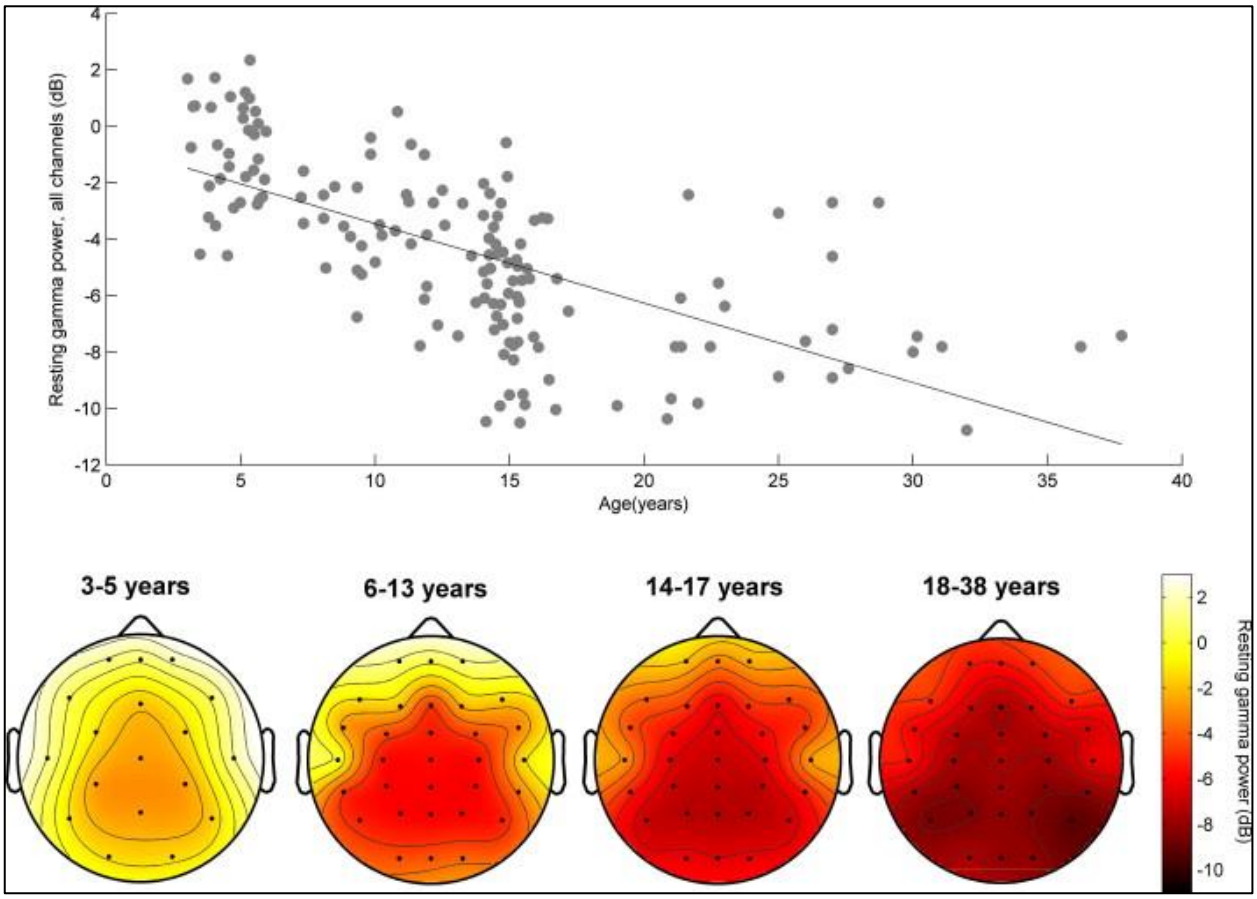


Figure 79: Resting absolute gamma power decreases with age in healthy controls. Top: Resting gamma power (31-50Hz) across all channels has an inverse relationship with increase in age. Bottom: Gamma activity is evenly distributed over the scalp with large decreases in power occurring in the post central regions with age. Extracted from Tierney et al (2013)

7.4.4 **Limitations**

There were several limitations to this study:

7.4.4.1 *Reliance on EEG reports and clinical recordings.*

A significant limitation on the study was the reliance on medical records and clinical EEG recordings. Medical records were difficult to obtain access, and the earliest EEG recordings close to the onset epilepsy were rare. Despite this, the detailed EEG reports were a good way of identifying whether the participant had features typical of RE. Furthermore, spike lateralisation was defined by experts in their field, which reduced error in interpretation. The expert information also provides greater certainty that the new generalised spike and wave discharges in follow-up were unseen in active epilepsy. The use of clinical EEG also had some benefits.

The use of clinical EEG in this study was complicated by; a small sample size, short periods of uncontrolled resting-state EEG and signal artefacts. The small sample size reduced the studies power, which means this study should be treated as pilot data. Therefore, this study needs to be repeated with a larger data set and complex modelling of the longitudinal data. Despite being a small sample size, the majority of the clinical EEG recordings contained high quality, artefact free resting-state EEG. There is a question about whether the resting state of clinical EEG constitutes a true resting state. This is because some authors define resting-state EEG as a non-task, which is preceded by standardised instructions and similar pre-experimental procedures (van Diessen *et al.*, 2015). This study does not fulfil this criterion, and therefore, the author recommends that the term “clinical resting state” should be used to differentiate the difference with other resting-state studies. Using clinical EEG for a resting-state study has many caveats; nevertheless, this study demonstrates that a small amount of clinical resting-state EEG can be used in a cross-sectional manner to measure changes in EEG power. Potentially this makes clinical resting-state EEG a valuable resource, which could drive the development of sophisticated tools for clinical EEG analysis.

7.4.4.2 *Altered recording parameters at follow-up and its effect on gamma*

At the follow-up, the EEGs were recorded using a high-density electrode array within a room electrically isolated within a Faraday cage. Electrical isolation is a unique feature which is not routinely used in clinical settings and may have had an influence on the recording of high frequencies in particular gamma. Electrical interference differences between baseline and follow-up are unlikely as the alternating current frequency in the UK is at 50 Hz, and the gamma band explored was between 30-50Hz. The level of vigilance may be another problem as the individuals at follow-up were mildly sleep-deprived, and this may have influenced the extracted power spectra. Altered EEG spectra is a possibility; however, this is unlikely to be different to the children with RE with active epilepsy are quite likely to have sleep deprivation as a result of their epilepsy (Tang *et al.*, 2011), so it is possible that the follow-up condition is comparable to baseline.

7.4.4.3 *Short periods of resting state*

Ten seconds of resting-state was EEG were used in this study, and this was not sufficient for obtaining a detailed analysis of resting state. This is because the EEG features may not be uniformly presented across the recording. Nevertheless, identifying good resting state in the clinical recordings was difficult due to spikes, sleep states and activation techniques such as photic stimulation and hyperventilation. Furthermore, 10 seconds of resting-state EEG has been used to as an alternative means to bio-metrically identify individuals with an 89% recognition rate in eyes closed epochs (De Vico Fallani *et al.*, 2011). Overall, the sampling of the EEG could have been better; however, the addition of a control group would have led to a better understanding of the data.

7.4.4.4 *Possible EEG artefact contamination*

There is a possibility that the EEG was marred by either slow eye movements which would be recorded as delta frequencies or electro-myographic activity which would be recorded as high frequencies components greater than 15 Hz. This problem was minimised by carefully checking each of the 10 second resting-state EEG sections and rejecting epochs which contained the aforementioned artefacts.

7.4.4.5 *No comparative control data*

This study would have benefited from having control data. Four controls EEGs were collected at the baseline, but due to time and resource limitations, follow-up recordings were not collected. The style of the investigation in the RE EEG data involved a mixture of cross-sectional and longitudinal datasets to explore changes in resting-state EEG across the ages of 8-14 years. Therefore, an expansion of this study would be to use control cross-sectional EEG data from a public EEG database such as the Child Mind Institute (http://fcon_1000.projects.nitrc.org/indi/cmi_eeg/eeg.html) to create a comparative group. The inclusion of this data set would provide greater insight into the changes seen in the resting state EEG between active epilepsy and seizure remission.

7.4.4.6 *Poor spatial localisation of EEG*

The topographic analysis of the resting state EEG data is sub-optimal. To improve the localisation and obtain the best interpretation, a source localisation process such as eLORETA software (Pascual-Marqui *et al.*, 2011) is required. The results could then be constrained to a model of the participant's cortex derived from computational neuro-imaging using Freesurfer; the individuals could then be collated into one group using a fixed standardised template (Fischl, 2012). This detailed method would reveal better insights. Nevertheless, the topographic technique is simple to use, easily

reproducible and utilises open-source software, which means this study could be replicated with little complication.

8 Conclusions

8.1 Overview

The thesis was designed to investigate what cognitive problems are apparent in individuals with RE during active epilepsy and whether they persist into seizure remission. To explore the substrates behind cognition, structural magnetic resonance imaging (MRI) and electroencephalography (EEG) were used at baseline and follow-up. This section will present a brief overview of the findings of each chapter.

8.1.1 Disordered cognition in RE is multi-layered, and deficits remain in seizure remission

The review of cognition in RE and other idiopathic epilepsies revealed little difference in cognitive problems between the two groups, furthermore interesting relationship between cognition and spikes and seizures were found. The review found no strong evidence for spikes or seizure influencing problems in core cognitive skills. Moreover, there was a lack of evidence to support transient cognitive impairment time-locked to spikes discharges. Nevertheless, there is evidence to support attentional improvements when spikes are in remission. As spikes are a marker of a hyper-excitable cortex, it would suggest when the cortex is in a hyper-excitable state, it can have an impact on the global function of cognition and behaviour. Seeing spikes as a marker of hyper-excitability rather than spikes being the cause of the dysfunction would be a reinterpretation of current thinking. Familial cognition matters as it would appear that a similar cognitive picture survives into seizure remission. This cognitive profile is closely related to dyslexia with problems with language, reading, motor problems and verbal memory. Overall, the review implied a layer of epilepsy-specific cognitive problems which remit with seizure remission to leave familial related cognitive problems.

8.1.2 A systematic review of neuroimaging reveals evidence for a disorder of neurodevelopment

A systematic review of neuroimaging studies found altered regions of grey and white matter in individuals with RE compared to controls. Measures of differences in grey matter volume and thickness were diffuse and outside of the central sulcus. These had a predominance within the frontal and parietal lobes. Subcortical grey matter structures were also enlarged, and these included the putamen, caudate and amygdala. In particular, there was a large amount of evidence for bilateral putamen enlargement compared to controls. White matter studies which investigated mean diffusivity (MD) and fractional anisotropy (FA) mainly found evidence of increased MD and decreased FA in the left hemisphere. This involved regions of the left superior longitudinal fasciculus, anterior thalamic tracts and connections between the pre and post-central gyri. The sole longitudinal study in the review revealed, reduced thinning of the cortex in individuals with RE compared to control. Furthermore, patches of thinner cortex at baseline become thicker over two years within the same regions. In subcortical structures, there was an increase in the volume of the bilateral putamen. Overall, the systematic review produced evidence to support a disorder of neurodevelopment and possible evidence of epilepsy influencing subsequent development.

8.1.3 Disordered cognition can appear or remain impaired in seizure remission

This chapter demonstrated a prevalence of cognitive problems within groups of individuals with RE, which is in keeping with the conclusions of the review in cognition. Developmental coordination disorder (DCD) appears to be the most prevalent of the cognitive problems with strong overlaps with dyslexia and attention deficit hyperactivity disorder. These problems can be identified during active epilepsy but can also surface in seizure remission. Indeed, some components of fluid intelligence/executive function appear to decline with age. Seizure remission generally improves cognitive abilities, for example, hyperactive behaviour and a specific auditory deficit, for filtered words.

However, additional testing in seizure remission identified specific problems with which can be seen in individuals with dyslexia, development coordination disorder or remitted developmental language disorder.

8.1.4 **Longitudinal imaging revealed abnormal development of brain structure.**

The neuroimaging analysis found evidence of aberrant development in individuals with RE in both cross-sectional and longitudinal analyses, which is in keeping with the conclusions of Chapter 2. The apriori hypothesised cortical regions demonstrated no difference at either baseline or follow-up, in seizure remission, whereas differences were seen in the vertices-based analysis at both time-points in the bilateral frontal lobes. In seizure remission, an additional area of thicker cortex was seen around the right central sulcus and posterior central gyrus. Longitudinal analysis revealed patchy cortical thinning of the cortex compared to controls. Where thinning occurred, this was in excess with clusters around the left lateral orbital frontal, superior frontal and pre-central regions compared to the control group. Differences were also seen outside of the neocortical layer.

Differences between the RE group and controls were seen in intracranial volume (ICV) and subcortical volumes. ICV and non-grey and white matter volumes, suspected to be largely influenced by ventricle volume, were found to be significantly different at both time-points. Longitudinally, both ICV and white matter volumes increased with age and may play a role in seizure remission. Similarly, there was an increase in the volume of the bilateral putamen volume with seizure remission, which replicates the development of the putamen in the Garcia-Ramos study (Garcia-Ramos *et al.*, 2015).

8.1.5 Brain activity changes in seizure remission but can contain still abnormalities several years after seizure remit

Qualitative and quantitative analysis of scalp EEG data revealed many changes in between active epilepsy and seizure remission. In active epilepsy, spikes were prevalent, but evidence of them are not required to have seizures. Furthermore, the temporal association between spikes and seizures was not well defined. Spikes could be apparent before the generation of seizures and were seen in individual in seizure remission up till the age of 15 years. A novel finding was the appearance of poorly formed generalised spike and wave discharges in seizure remission. The quantitative analysis revealed changes with seizure remission.

The quantitative analysis demonstrated a significant reduction in power and a significant increase in dominant global frequency. The changes in dominant global frequency did not correlate with the time to final seizure, whereas specific frequency power components had a relationship. There was a correlation between time to final seizure and a decrease in relative delta power. The decrease in relative delta power was correlated with an increase in relative beta power. Furthermore, spatial analysis of absolute power across the scalp between the two time points revealed a reduction of delta activity over the midline and a large decrease in generalised gamma activity.

In summary, this thesis has demonstrated many differences in the brain structure and function and cognition of individuals with RE compared to healthy controls. Some of these features are apparent in both active epilepsy and seizure remission the following section will explore the implications of this thesis.

8.2 Strong evidence of neuro-developmental origins

There is strong evidence that RE is a result of disorder neurodevelopment. The cognitive evidence indicates that co-occurring cognitive neurodevelopmental disorders are prevalent. In particular, developmental coordination disorder (DCD), developmental language disorder (DLD), dyslexia and attention deficit hyperactivity disorder (ADHD). Furthermore, the large overlaps between cognitive profiles have similarities with children with these disorders without seizures and are keeping with the procedural deficit hypothesis (Ullman, Michael T. and Pierpont, Ullman and Pierpont, 2005) which explains cognitive overlap in individuals with neurodevelopmental disorders. The neuroimaging evidence suggests larger intracranial volumes and an increase non-grey/white matter volume which most likely indicates enlarged ventricles, which can be seen in individuals with neurodevelopmental disorders (Lyoo *et al.*, 1996; Piven *et al.*, 1996). Furthermore, there appears to be multiple patches of both thick and thin cortical regions predominantly within the frontal lobe. This is the lobe which is highly associated with developmental disorders of the brain. The EEG data would suggest that there is possibly an excess of delta waves activities in children with RE with an emphasis along the midline. Furthermore, there is evidence that an individual can generate Rolandic spikes as young as two years of age without experiencing seizures. Delta activities and RS have both been associated with disorders of neurodevelopment. Overall, this thesis has found a collection of evidence which supports the hypothesis that RE is a seizure disorder which is associated with disorders of neurodevelopment. In seizure remission, this is associated with significant changes in brain structure and function, yet evidence of altered neural development persists. The altered development of the brain in individuals with RE could be related to the over-expression of PAX6.

8.3 The potential role of PAX6: A hypothesis

In keeping with the neurodevelopmental evidence, this thesis has produced evidence which is in keeping with the findings of in-vitro and in-vivo models of pax6 overexpression. There appear to be both regions or thinner and thicker cortex which may have been influenced by thinner cortical layers (Georgala, Manuel and Price, 2011) and a lack of white matter maturation due to insufficient oligodendrocytes (Jang and Goldman, 2011). Furthermore, in PAX6 over-expression models, there is an excess of pyramidal cells at the expense of interneurons (Sansom *et al.*, 2009) which results in an increase in cortical excitation provided by an excess of pyramidal cells and reduced inhibition provided by a reduction in interneurons (Markram *et al.*, 2004). The lack of myelination of pyramidal axons may also play a role in increasing excitability (Merkler *et al.*, 2009). The reduction of cortical myelination could have two effects: one, the processing of information and subsequent cognition is altered as there is an imbalance between excitation and inhibition (Rubenstein and Merzenich, 2003) and two the imbalance makes the cortex hyper-excitable and thus susceptible to developing intermittent inter-ictal discharges (Kellaway, 2000) a marker of cortical hyperexcitability. The changes which occur with seizure remission result in a thinning of the cortex with this process, reducing the excitability, as seen in the remission of spikes and seizures. Furthermore, there is an improvement in cognition with seizure remission implicating that the hyper-excitability is part of the cognitive dysfunction. It is proposed that the remaining cognitive dysfunction is due to the residual neuro-developmental problems that were laid down by PAX6 but are not adequately masked by changes in brain structure during the time period when seizures remit. This raises questions about whether RE can become refractory due to atypical brain development during adolescence and whether the brains of individuals with RE as child respond differently to normal ageing in later life.

8.4 Cognition in seizure remission co-occurs with changes in brain structure and function.

This thesis demonstrated that overall, there is an improvement in cognitive function in seizure remission, which appears to occur in conjunction with changes in brain structure and function. This longitudinal neuroimaging analysis would suggest that a decrease in cortical thickness is beneficial to the improvement in cognitive function. Similarly, changes in the electroencephalogram (EEG) would suggest that a reduction in spikes, absolute signal power and relative delta power are required for an improvement in cognition. Altogether these findings would suggest that measures of neural maturation are associated with improvements in cognition. These markers of maturation would also suggest that they are driving the remission of seizures.

8.5 Evidence that changes in brain function and structure could drive seizure remission.

The EEG analysis found that that there was a relationship between global relative delta power and the time to final seizure would suggest that this is driving force behind the remission of seizures. As mentioned in the EEG chapter, recent evidence has suggested that there is a relationship between grey matter volume and delta power. It could be the case that the decrease in cortical thickness is associated with the change in delta EEG power. In the MRI chapter, there is longitudinal evidence of increased cortical thinning over the left superior frontal region and left pre-central regions. These regions seem to co-occur with where there are topographic changes in delta activity between baseline and follow-up. The overlap between cortical thickness change and change in topographic delta activity could be an indicator of the regions involved in the generation of seizures in RE.

8.6 Residual cognitive problems in seizure remission and the relationship with brain structure and function.

In seizure remission, there is evidence of both persisting and worsening cognitive problems. The persisting cognitive problems seemed to fit within the definition of dyslexia, which includes problems with phonological processing, reading, language and verbal memory. The persisting structural problems were seen at follow-up were at global, cortical and subcortical levels. At the global level, there was evidence of an enlarged ICV, at the cortical level the left middle rostral frontal region was thicker than controls and at the subcortical level, non-grey or white matter volumes were enlarged, a possible proxy of ventricular enlargement and the bilateral putamen were enlarged. Whether these changes relate to persisting cognitive problems is unclear; however, a recent imaging study has identified abnormalities within the left middle rostral regions of children with developmental dyslexia.

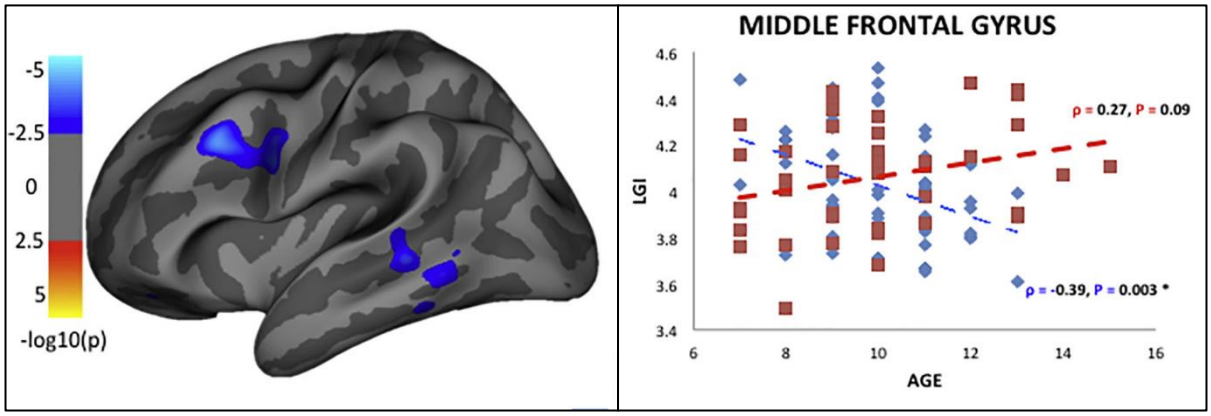


Figure 80: Left hemisphere local gyrification index analysis comparing healthy subjects to those with developmental dyslexia. Left: clusters of regions with statistically significant difference in correlation between LGI and age compared to controls. Clusters are overlaid on an inflated brain hemisphere in lateral view. The map is -log10(P), where P is the significance. A Min of 2 will display all vertices with P < 0.01 and a Max of 5 will show vertices of P < 0.0001 as the same colour. Right: Scatterplot of the middle frontal gyrus cluster. X axis: Age, Y-axis: LGI. Red squares: Developmental dyslexia, Blue squares: Healthy controls.

In a study of a correlation between gyrification and neurite density and orientation dispersion with age between individuals with developmental dyslexia and healthy controls, the left middle rostral frontal region was found to be statistically different (Figure 80). Furthermore, there was no age-related correlational decrease in the local gyrification index (LGI), which equates to a reduction in the

invagination of cortical surface which should result in normal development (Caverzasi *et al.*, 2018). When the LGI measure was compared with values of orientation dispersion, there was a positive correlation (Caverzasi *et al.*, 2018). The orientation dispersion (ODI) value is a measure of how closely packed and aligned the neurons are; an increase in ODI with age would suggest a dispersion of neurons and lack of myelination (Zhang *et al.*, 2012). Indeed, a study in white matter in RE identified similar regions, Ciumas et al. found a region in active epilepsy (MNI coordinates: x −34, y 36, z 16) in the left middle frontal gyrus with increased MD compared to controls (p=0.011), Interestingly, the Ciumas regions coincides with a similar area of cortical thickening (x -41.7,y 30.6, z 20.1, p=0.0024) in this study presented in MRI chapter (Ciumas *et al.*, 2014). The overlaps between the three studies provide a strong hypothesis for further investigation of the region in individuals with dyslexia and in family members of children with RE. Some of the persisting cognitive problems appear to involve fine motor skills.

In seizure remission, there was a deficit in fine motor skills identified by the grooved pegboard task. The deficit was seen in both hands but worse in the non-dominant (p=0.038, d=0.86), 94.1% were right-handed at follow-up, so this would indicate the left hand. Also, at follow-up, a cross-sectional analysis found an increase in cortical thickness with age in the RE group compared to controls. This increase was over the lower post-central gyrus and sulcus, the supramarginal gyrus, transverse temporal gyrus and the upper portion of the superior temporal region. It could be possible that this thickening of the cortex, particularly of the primary sensory and association cortices is influencing the ability to perform fine motor tasks. Furthermore, this region coincides with the area where rolandic spikes are recorded. Interestingly, analysis of the clinical reports identified a predominance of spikes over the right hemisphere which raises the question of whether spikes are generated in regions likely to thicken with age or does spiking lead to the increased cortical thickness and whether this is related to impaired left-hand motor function. Another deficit that is apparent in seizure remission is transient.

At follow-up, abnormal auditory processing was reported in 23% of the participants with RE in seizure remission. The processing problem was detected using the gaps in noise test, which would suggest that there is evidence of an intermittent transient disorder of temporal auditory processing. Interestingly, this may have been demonstrated in a recent study (Amaral *et al.*, 2015) where there was suspicion of seizure remission in the cohort. The cause of this dysfunction could be Rolandic

spikes (RS), but this is unlikely, because as mentioned in Chapter 2, there is no strong evidence to support transient cognitive impairment time-locked to RS, however it is important to note that this author could not find any TCI studies which used an auditory task individuals with RE (Aldenkamp and Arends, 2004). Nevertheless, the appearance of generalised spike and wave discharges in the EEG chapter would raise the possibility that these inter-ictal discharges can transiently influence cognition. Indeed, generalised spike and wave discharges are more likely to induce TCI (Binnie, 2001) and impaired consciousness and have been recognised to alter auditory perception (Nicolai *et al.*, 2012).

Overall there are many overlaps between the cognitive findings, neuroimaging and EEG findings in individuals with RE in seizure remission. These have many implications.

8.7 Educational psychology assessment and support is required for individuals in active epilepsy and seizure remission.

The findings of this study would suggest that the prevalence of cognitive problems in individuals with RE has policy implications. It would seem prudent to assess an individual with RE once they had obtained their diagnosis, and this should involve assessments for all the main SPLDs, which include, DCD, DLD, Dyslexia and ADHD. In particular, assessment should focus on fine motor skills, phonological and auditory processing and attentional problems. Single-word reading and rapid letter naming, which is commonly used tests to assess reading ability appear to be insensitive to problems in individuals with RE, suggesting a dyslexia variant. This testing should also be repeated in seizure remission.

The evidence of this thesis would strongly recommend the repeat of neuropsychological testing at seizure remission in individuals with RE. Interestingly, a recent report in absence epilepsy has identified the same problems (Fonseca Wald *et al.*, 2019). Currently, the National Institute for Clinical Excellence (NICE) guidelines support children with epilepsy and advises an agreed and comprehensive epilepsy care plan (NICE, 2013). In their definition of a care plan, it includes any issues regarding “education and healthcare at school”. The problem is recent definitions of epilepsy and care for epilepsy requires the child to be having or at risk of seizures (Fisher *et al.*, 2014). Once seizures remit, the package of care is withdrawn, and children with the cognitive aspects of the epilepsy are left with little support.

Overall, the results of this thesis would suggest that educational neuro-psychological testing should be part of the routine care package at diagnosis and in confirmed seizure remission.

8.8 Further investigations

This thesis has produced many new avenues of investigation. These new avenues are in all of the methodologies used. This thesis has demonstrated changes in cortical thickness and cortical volume between active epilepsy and seizure remission. There is clear evidence of change; however, it is unknown what drives this and whether variation in this change alters the cognitive outcome.

One particular puzzle is, what do the measurements of cortical thickness represent? As mentioned in the MRI chapter of this thesis, there is a debate about what MR measurements of cortical thickness and subcortical volume represent. To fully investigate these processes requires exploring the existing MRI data in relation to surface area (Winkler *et al.*, 2018) and local gyrification index (Schaer *et al.*, 2012). In addition to the collection of extra MR scan protocols such as diffusion-weighted images (Yoshida *et al.*, 2013) in particular neurite orientation, dispersion and density imaging (NODDI) (Zhang *et al.*, 2012). The use of these extra techniques would allow for a better understanding of changes to the cortical tissue at a microstructural level and may be able to separate whether the cortical thickness is changing due to cortical processes such as synaptic pruning, myelination processes, changes in sulcal shape or orientation of pyramidal cells. How these measures are associated with resting EEG data is particularly pertinent.

In the EEG chapter, one of the unexpected findings of the study was a relationship between global relative delta and the time to final seizure, which diminishes the role of the Rolandic spikes in the generation of seizures. Furthermore, in the MRI chapter, alterations in cortical thickness were demonstrated. Using the work of Whitford *et al.* it was hypothesised that this change was due to a reduction in cortical thickness (Whitford *et al.*, 2007). This apparent relationship needs to be explored in much greater detail because if there is a strong association between cortical thickness and absolute delta wave power, it could be used as a tool. This tool could be used to monitor the development of the child with epilepsy and possibly predict the time to seizure remission. A driver of the change in cortical thickness and delta activity could be driven by developmental changes during puberty.

A driver of this change could be puberty. All of the RE cohort were in seizure remission at follow-up but was this driven by puberty? There is lots of evidence detailing large changes in brain structure in adolescents which occur during puberty and are associated with an increase in sex hormones (Blakemore, Burnett and Dahl, 2010). The individuals with RE in this study had varying points of seizure remission. However, it would be interesting to understand if seizure remission correlated with signs of puberty and does this relate to the sex of the individual, this is an interesting hypothesis, which is worth further exploration. Indeed, does the change that occurs in brain structure within an individual have an effect of cognitive ability in seizure remission.

The relationship between brain structure and cognitive ability would be an enlightening avenue of investigation. It is unknown, how much thinning of the cortex is required and where it occurs in the brain is needed to improve cognitive abilities. Finally, in individuals with residual cognitive problems in seizure remission, does their severity of the deficit correlates with brain structure, for example, the thickness of the inferior frontal gyrus and phonological processing ability? Furthering knowledge in this area would allow for the monitoring of the development of cognitive abilities using MR techniques in individuals with RE and tool, which is currently lacking. A possible confound of the relationship between cognition and brain structure is AED therapy.

Another unexplored area was the use of AED therapy in cognitive outcomes. In RE, seizure remission is a near possibility, regardless of the use of AED therapy. Nevertheless, an interesting investigation would be to see if the use of AEDs resulted in a better or worse cognitive outcome? Furthermore, does the use of AEDs affect the measured changes in brain structure and function? Overall, this information could be used to determine what is the AED of choice for the best cognitive outcome.

In summary, this thesis had raised a multitude of questions; some are refining the original methodologies of this study, whereas others propose new investigations to further understanding of brain structure and function in RE.

8.9 Final statement

This thesis has performed a detailed longitudinal study of brain structure and function in individuals with RE. The cognitive testing and neuroimaging have revealed many differences with healthy controls suggesting an alteration in brain development, which is mitigated with changes in brain development during the period of adolescence. In addition, brain function dramatically changes, and there is a risk of developing new epileptiform abnormalities. It is hoped that this thesis will add productively to the debate on cognition in RE, and it will encourage new ways of thinking about Rolandic epilepsy. Finally, the take-home message of this thesis is that cognitive problems, which require educational support are prevalent in both active epilepsy and seizure remission and suitable arrangements should be made for individuals with RE to help them prosper and succeed as children and adults.

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10 Appendices

10.1 Systematic review of neuroimaging

Cingulum structures: Cingulate gyrus and Cingulum Hippocampus
Corpus Callosum: Body of corpus callosum, Genu of Corpus Callosum, Splenium of corpus callosum, Tapetum
Corticospinal tract: Corticospinal tract
External capsule
Fornix: Fornix column and body and Fornix stria terminalis
Inferior network: inferior fronto-occipital fasciculus (IFO), sagittal striatum including inferior longitudinal fasciculus (ILF) and Uncinate fasiculus
Internal capsule and thalamic radiations: Anterior Corona Radiata, Posterior corona radiata, Superior corona radiata, Anterior limb of the internal capsule, Anterior thalamic radiation, Posterior thalamic radiation including optic radiation Posterior limb of internal capsule and Retrolenticular part of the internal capsule
Mid brain, brainstem and cerebellar tracts: Cerebral peduncle, Middle cerebellar peduncle, Inferior cerebellar peduncle, Superior cerebellar peduncle, Pontine crossing tract, Medial lemniscus
Perisylvian networks: Superior fronto-occipital fasiculus and Superior longitudinal fasciculus

Figure 81:John Hopkins University atlas used in Tract Based Spatial Statistics

10.2 EEG in RE between active epilepsy and seizure remission

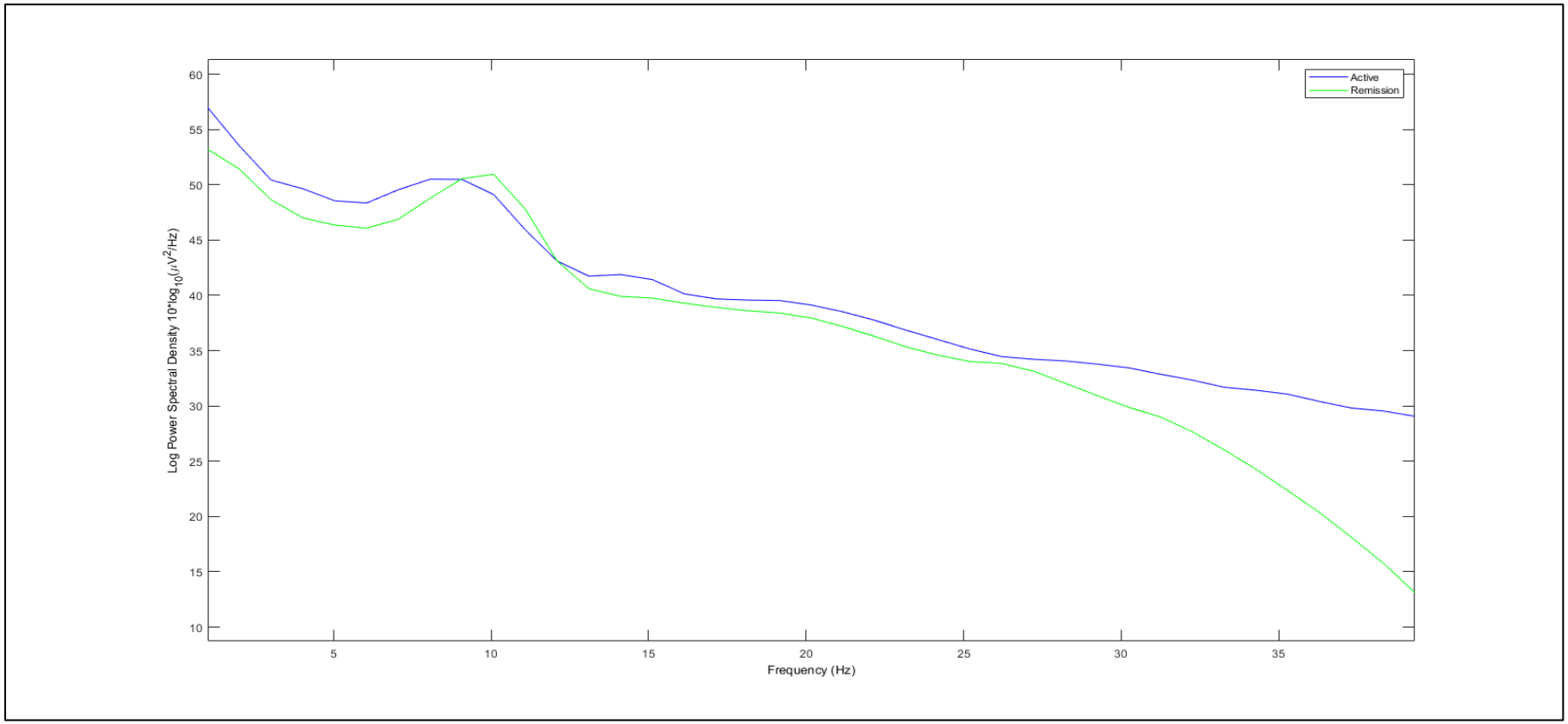

Figure 82: Average EEG power spectrum of individuals with Rolandic epilepsy between active epilepsy and seizure remission. An averaged EEG spectra of 19 electrodes uniformly distributed across the scalp. Collected from 6 participants with active Rolandic epilepsy (blue) and thirteen in seizure remission (green). The spectrum (x axis) ranges between 0.5 and 45 Hz. Left to right active epilepsy (blue line): The delta frequencies have the highest power which steeply reduces between 0.5-3 Hz but is more gradual between 3-6 Hz. At 6 Hz, there is an increase in power which leads to a peak of the dominant frequency at 9 Hz. A small secondary peak is seen between 13-16 Hz, this is followed by a reduction in absolute power with an increase in frequency. Left to right seizure remission (green): Compared to active epilepsy there is a reduction in delta and theta power however, there is a similar steep decline in power in both the delta and theta frequency bands. At 4 Hz the decrease in power becomes gradual and plateaus. The peak of the dominant frequency is 10 Hz which is faster than the peak in active epilepsy, the peak also had increased power. After 12 Hz there was a reduction in power and this is particularly prominent after 27 Hz, part of the beta frequency band. At the end of the spectra >24 Hz there is a large drop in the power of these frequencies compared to active epilepsy.

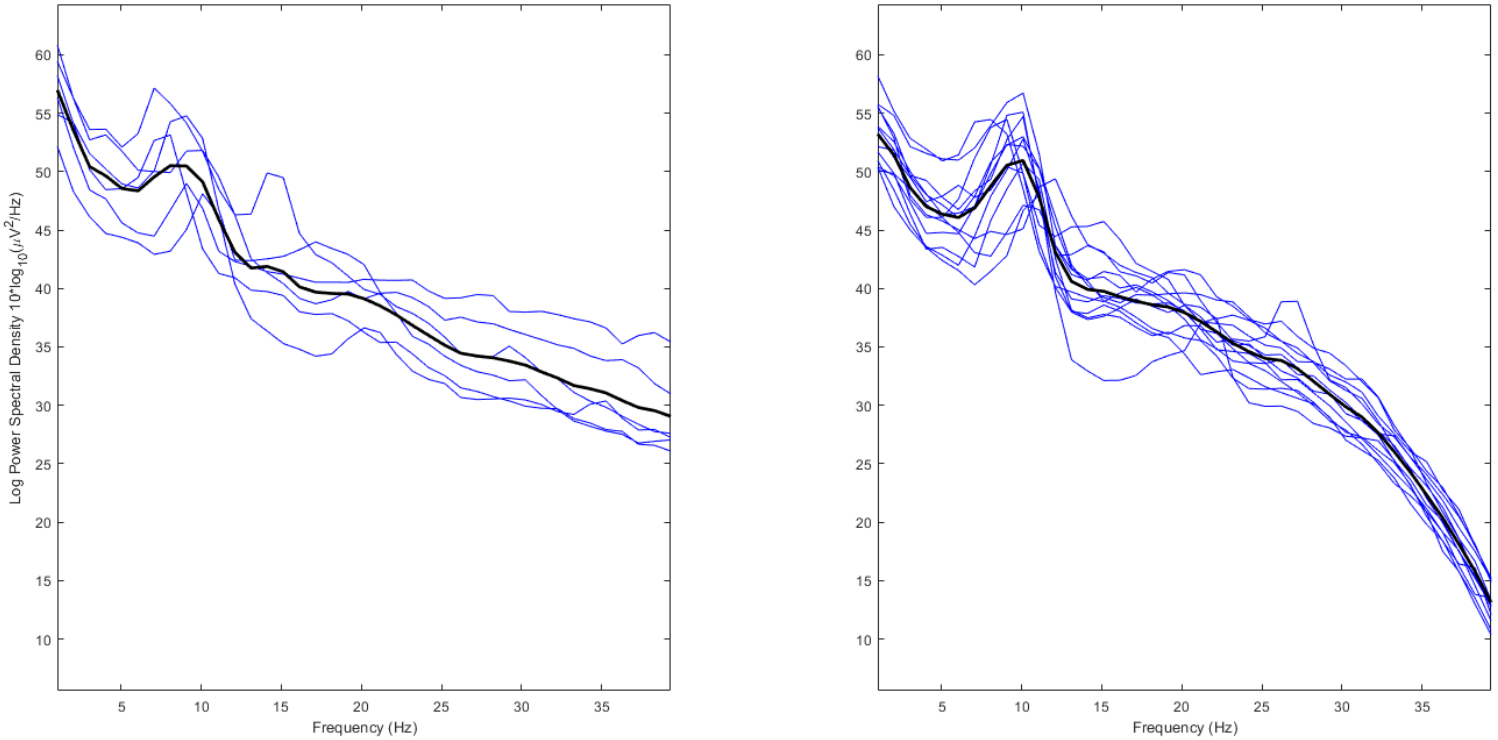

Figure 83: Individual resting state spectra between active epilepsy and seizure remission. Six participants with active Rolandic epilepsy (left panel) and thirteen in seizure remission (right panel). The spectrum (x axis) ranges between 0 and 35 Hz. Global spectrum collated from 19 electrodes uniformly distributed across the scalp. Average spectrum (black), Individual spectra (blue).